REGULATION OF SECRETION OF PARATHORMONE AND SECRETORY PROTEIN-I FROM SEPARATE INTRACELLULAR POOLS BY CALCIUM, DIBUTYRYL CYCLIC AMP, AND (1)-ISOPROTERENOL

JEREMIAH J. MORRISSEY and DAVID V. COHN

From the Calcium Research Laboratory, Veterans Administration Medical Center, Kansas City, Missouri 64128, and the Department of Biochemistry, University of Kansas Medical Center, Kansas City, Kansas 66103

ABSTRACT

Dispersed porcine parathyroid cells were incubated at calcium concentrations between 0.5 and 3.0 mM in the presence of 3H- or 14C-amino acids to label newly synthesized parathormone. Up to four times more hormone was secreted at the lower calcium concentration but its specific radioactivity, from 30 to 50 times that of the intracellular pool, did not change. Dibutyryl cyclic AMP doubled immunoactive parathormone secretion at each calcium concentration, but there was no increase in secretion of radioactive hormone if labeled amino acids and secretagogue were added simultaneously. Similarly, when the intracellular pool of parathormone was prelabeled with 3H-amino acids and then the cells were incubated in 14C-amino acids and dibutyryl cyclic AMP, the entire increase in hormone secreted was derived from the prelabeled pool. (1)-isoproterenol increased intracellular cyclic AMP and acted on hormone secretion in a manner indistinguishable from dibutyryl cyclic AMP. In similar double-label experiments dibutyryl cyclic AMP preferentially enhanced secretion of secretory protein-I, a calcium-regulated protein of the parathyroid of unknown function. Calcium, alone, inhibited the intracellular level of cyclic AMP in a concentration-dependent fashion. These data are consistent with the existence in the parathyroid cell preparation of two hormone and secretory protein pools that may be individually recruitable—one consisting of most recently synthesized protein, the other consisting of older “storage” protein. The data do not allow one to decide whether the two pools coexist within individual cells or whether, instead, they exist in separate cells of the dispersed gland preparation.

KEY WORDS parathormone · secretory protein-I · cyclic AMP · protein secretion · (1)-isoproterenol · calcium

The primary physiological regulator of parathormone secretion is ionized calcium (12, 27, 28, 38). Several other agents also affect its secretion, and their potential roles as physiological modulators of secretion have been considered. These agents include: Mg²⁺ (7, 27, 37), β-adrenergic catecholamines (4, 6, 15, 39), dopamine (2), α-adrenergic catecholamines (5), prostaglandins of the E series.
(16), dibutyl cyclic AMP \(^1\) (1, 39), and vitamin D metabolites (8, 14). Cyclic AMP appears to play a critical role in the secretory process. In most cases in which enhanced secretion of hormone has been elicited, including hypocalcemia, the cyclic AMP level within the parathyroid cell has also been elevated (1, 4, 11). Finally, the parathyroid has been shown to contain a calcium-inhibited adenylate cyclase (13, 22, 31).

The fundamental mechanisms through which these various secretagogues and cyclic AMP function in the parathyroid are not understood, in part, because the secretory pathways in the gland have only recently come under detailed study. During the processing, packaging, and secretion of parathormone, newly synthesized hormone is, for the most part, degraded and the remainder is either stored (9, 10, 17). Earlier data from this laboratory suggested that the gland contains two hormone pools that may contribute to secretion: one consisting of newly synthesized hormone, and the other a slowly equilibrating pool of stored hormone, presumably in secretory granules (21). In in vitro studies with parathyroid tissue slices there appeared to occur a preferential secretion of more recently synthesized hormone compared to that from the storage pool. When hormone secretion was stimulated by decreasing the concentration of calcium in the medium, the proportion of hormone contributed from the newly synthesized pool was somewhat greater.

In none of the previous studies were the possible differential effects on secretion of the various secretagogues tested. In the present study, therefore, using a dispersed parathyroid cell system (3, 24, 25), we compared the relative ability of calcium, dibutyl cyclic AMP, and (1)-isoproterenol to stimulate secretion of hormone from the putative newly synthesized and storage pools of parathormone. Under similar conditions, we also examined the secretion of secretory protein-1, a parathyroid protein of unknown function (19, 24, 25) whose release from the gland closely parallels that of parathormone.

**MATERIALS AND METHODS**

Proximal parathyroid cells were prepared by the collagenase-DNase procedure of Brown et al. (3) with slight modification (25). In all experiments the cells were incubated at a density of 4 \(\times\) 10\(^5\) cells per ml in Krebs-Ringer supplemented buffer (25) at 37°C containing 1 mM magnesium sulfate and the indicated concentration of calcium chloride. Dibutyl cyclic AMP and (1)-isoproterenol, when added, were used at 1 mM and 1 \(\mu\)M, respectively. In the initial experiments, the cells were incubated with 50 \(\mu\)Ci/ml \(^3\)H-amino acid mixture (TRK 440, Amersham Corp., Arlington Heights, I.U.) for 90 min.

Viability of the dispersed cells was assessed by trypan blue exclusion and was found to be in excess of 90%. In addition, the cells were incubated for 20 min with \(^{35}\)S)methionine, after which they were chased with non-radioactive methionine for an additional 20 min, washed, then fixed and examined by light microscope autoradiography (29). The bulk of the cells contained about the same level of radioactivity (Fig. 1).

In pulse-chase experiments designed to differentially label the pools of parathormone available for secretion, the cells were incubated at 3.0 mM calcium chloride and 100 \(\mu\)Ci/ml \(^3\)Hlysine (90 Ci/mmol) for 60–90 min followed by a 60-min chase in the presence of 250 \(\mu\)M nonradioactive lysine. During the chase period, the incorporation of \(^3\)H label into total protein ceased. The cells were harvested by gentle centrifugation and resuspended in fresh medium containing 5 \(\mu\)Ci/ml \(^{14}\)C]leucine (354 mCi/mmol) at the indicated calcium and secretagogue concentrations. Under these conditions, the most recently synthesized protein contained \(^14\)C label.

Since the standard buffer system contained only those amino acids of the serum and those released by the cells, it was possible that the rate of incorporation of radioactive amino acids was not maximal. To check this, we measured the incorporation of amino acid into cellular protein in the presence or absence of a full complement of added amino acids. In these studies, the rate of incorporation of \(^{3}\)H]leucine was the same whether or not a mixture of 19 unlabeled amino acids (250 \(\mu\)M each) was added to the incubation buffer.

Radioactive protein in the medium was precipitated with 25% trichloroacetic acid at 4°C. It was gathered by centrifugation and washed with 25% trichloroacetic acid and then with ether to remove the trichloroacetic acid and dissolved in 100 \(\mu\)l of 6 M urea-0.5 N acetic acid. We find that 25% trichloroacetic acid rather than the 10% used previously (24, 25) precipitates a greater portion of parathormone fragment. Cell pellets were resuspended into 2 ml of 8 M urea-0.2 M cysteine-0.1 N hydrochloric acid and homogenized with the aid of a Polytron (Brinkmann Instruments, Inc., Westbury, N. Y.). This procedure solubilized the entire cell contents.

Proteins in the homogenate were precipitated by the addition of an equal volume of 50% trichloroacetic acid and processed as described above for the protein of the media.

Radioactive proteins of the medium and of cellular origin were separated on 10% polyacrylamide gels utiliz-
FIGURE 1 Light microscope radioautography of radioactive amino acid-labeled porcine parathyroid cells. Cells were incubated with [35S]methionine and processed as described in Materials and Methods. Some cells appear devoid of grains due to the thickness of the specimen and limited focal plane. When these regions are brought into focus, autoradiographic grains are also seen above these cells. Cells were stained with hematoxylin and differentiated with dilute hydrochloric acid. Overall magnification is approx. 1,500.

Using the pH 4.5 buffer system of Reisfeld et al. (30) containing 6 M urea and a fourfold molar excess of N,N'-diallyltartardiamide in place of N,N'-methylenbis-acrylamide. The gels were frozen, sectioned into 1-mm segments with a Mickle gel slicer (Brinkman Instruments, Inc.), and dissolved samples were assayed for radioactivity by liquid scintillation counting in a toluene-Triton-based phosphor solution (18). Typical radioactive patterns for the separation by this technique for parathormone, parathormone fragment, and secretory protein-I are listed below in Fig. 2A.

Radioimmunoassay of parathormone in the spent incubation media and in extracts of the polyacrylamide gel slices was performed as described previously (18, 24) using goat antisera (G-1811) that recognizes the carboxy-terminal portion of parathormone.

For assay of intracellular cyclic AMP, the cell pellets immediately after centrifugation were extracted with 5% trichloroacetic acid. The acid extracts were then extracted with ether several times to remove the trichloroacetic acid and lyophilized to dryness. The residues were dissolved in 50 mM sodium acetate, pH 6.2, and the cyclic AMP content was determined by means of radioimmunoassay with cyclic AMP 125I tracer and cyclic AMP antiserum after acetylation of the sample. Overall recovery of cyclic AMP averaged 85%.

All radioactive amino acids were obtained from Amer sham Corp. Supplies necessary to conduct the radioim...
munoassay of cyclic AMP were obtained from New England Nuclear (Boston, Mass.). Assay of radioactivity was performed by liquid scintillation spectrometry. Other sources of reagents and other minor procedures have been previously described (24).

RESULTS

Table 1 lists the effects of dibutyryl cyclic AMP on the specific radioactivity of the secreted and intracellular parathormone at various calcium concentrations. The amount of both radiolabeled and immunoactive parathormone increased coordinately in the medium as the concentration of calcium was lowered. Thus, the specific radioactivity of the hormone in the medium remained the same. The amount of total cellular parathormone was up to 200-fold greater than that which was secreted. Its specific radioactivity was relatively the same at all calcium concentrations but was 30- to 50-fold less than that of the secreted hormone. Dibutyryl cyclic AMP stimulated secretion of parathormone about twofold at all concentrations of calcium. In contrast to the effect of calcium, this extra hormone was all nonradioactive. Therefore, on average, the specific radioactivity of the hormone in the medium in the presence of dibutyryl cyclic AMP was one-half that of the controls. Dibutyryl cyclic AMP had little or no effect on the tissue hormone level or its radioactivity.

We had one concern about the calculated values for specific activity of parathormone listed in Table I. The values were derived from the radioactivity in intact parathormone actually measured

TABLE I

| Ca | Control | + DBcAMP | Control | + DBcAMP | Control | + DBcAMP |
|----|---------|----------|---------|----------|---------|----------|
| mM | ng      | dpm      | ng      | dpm      | ng      | dpm      |
| A. Secreted | | | | | | |
| 3.0 | 4.5 | 8.9 | 5.000 | 5,400 | 1.185 | 675 |
| 2.0 | 5.1 | 11.6 | 6,200 | 6,440 | 1.180 | 700 |
| 1.5 | 11.1 | 23.3 | 10,950 | 11,870 | 990 | 510 |
| 1.0 | 16.0 | 29.7 | 18,900 | 20,680 | 1,215 | 555 |
| 0.5 | 16.8 | 30.4 | 19,900 | 20,485 | 1,100 | 610 |
| Average | 1,136 ± 90 | 610 ± 80 |
| B. Cellular | | | | | | |
| 3.0 | 890 | 890 | 20,170 | 20,090 | 22.7 | 22.6 |
| 2.0 | 910 | 880 | 19,030 | 19,510 | 20.9 | 22.2 |
| 1.5 | 890 | 850 | 17,250 | 16,940 | 19.4 | 19.9 |
| 1.0 | 810 | 850 | 16,250 | 16,010 | 20.1 | 18.8 |
| 0.5 | 850 | 830 | 15,780 | 16,380 | 18.6 | 19.7 |
| Average | 20.3 ± 1.6 | 20.6 ± 1.7 |

The cells were incubated in the presence of the 50 μCi/ml $^3$H-amino acid mixture for 90 min at the indicated calcium concentrations in the presence and absence of 1 mM dibutyryl cyclic AMP (DBcAMP). At the end of the incubation period, the cells and media were separated by centrifugation and processed for assay of immunoreactive and radioactive parathormone (PTH). Results represent the average of duplicate determinations.
after separation by polyacrylamide gel electrophoresis (Fig. 2) and the immunoactive "parathormone" of the medium which we showed earlier to consist of parathormone itself and some hormonal fragments (24). The calculated specific radioactivities, therefore, would have been affected should there have been a change in ratio of fragment to intact hormone in the samples. We ascertained that this was not the case by direct immunoassay of the parathormone and fragments in the polyacrylamide gels. Fig. 3 portrays the distribution of immunoactivity in incubation medium under the various experimental conditions. In accord with the data of Table I, there was more parathormone in the low calcium than high calcium medium, and at either concentration dibutyryl cyclic AMP enhanced secretion. The ratio of hormone to fragment peaks, however, under all conditions remained essentially the same, supporting the validity of the specific activity data of the table.

These data suggested that dibutyryl cyclic AMP preferentially stimulated secretion of the previously synthesized (i.e., nonradioactive) hormone of the cell. This was tested in an alternative way with parathyroid cells that had first been incubated with [3H]lysine or [3H]leucine for 90 min, chased for 60 min by incubation with nonradioactive amino acid, and finally, incubated with [14C]leucine or [14C]lysine in the presence or absence of dibutyryl cyclic AMP. This approach was designed to allow us to discern simultaneously the fate of radioactive parathormone made by the cell before and coincident with stimulation by dibutyryl cyclic AMP. Table II shows that the amount of total parathormone secreted was 3.5- to 4-fold greater at 0.5 mM calcium than at 3.0 mM calcium. At both concentrations, the hormone appeared to be derived from both the previously synthesized and more recently synthesized pools in about the same proportion. The increase in previously synthesized hormone secreted in response to the lower calcium concentration provides evidence that calcium affected secretion from both the newly synthesized and storage pools. Dibutyryl cyclic AMP doubled the amount of immunoactive hormone secreted, and this additional hormone was derived preferentially from the pool of older hormone since there was essentially no increase in release of 14C hormone but an increase of 40% in release of 3H hormone averaged for the two experiments. Because the percentage increase in total hormone was about twice that of radioactive hormone derived from the previously labeled pool, it would appear that the 3H parathormone had not fully equilibrated within the storage pool.

We earlier showed that the rates of synthesis of parathormone as well as total protein were unaffected by the calcium concentration in which the cells were incubated (9). In the present study, we confirmed this for calcium and also found that these parameters were unaffected by dibutyryl cyclic AMP. For example, in one of several such experiments, the incorporation of 3H-amino acids into parathormone at 0.5 mM and 3.0 mM calcium, with or without 1 mM dibutyryl cyclic AMP, averaged 36,300 ± 1,100 dpm per 10 min per 10^5 cells. The incorporation of radioactive amino acid into total cellular protein under the same conditions averaged 118,500 ± 10,900 dpm. There were no differences in either parameter due to calcium or dibutyryl cyclic AMP. These data together with those in Table I that show that the amount and specific radioactivity of intracellular parathormone did not change indicate that the effect of dibutyryl cyclic AMP on the amount of parathormone in the medium was exclusively on secretion.

Presumably dibutyryl cyclic AMP worked on the parathyroid cells by mimicking the action of an appropriate hormone that would produce an increase in intracellular cyclic AMP. We tested this supposition by examining the effect of the β-agonist, (1)-isoproterenol, a parathormone secret-
TABLE II

Effect of Calcium and Dibutyryl Cyclic AMP on the Secretion of Previously Synthesized ("Old") and Newly Synthesized Parathormone

| Exp | Condition | Immunoactive hormone | \[^{1}H\]-"old" hormone | \[^{14}C\]-new hormone |
|-----|-----------|----------------------|--------------------------|-----------------------|
|     |           | ng/2 h/10^6 cells    | dpm/10^6 cells           |                       |
| A.  | 0.5 mM calcium | 15.0 ± 1.0           | 5,100 ± 500              | 4,260 ± 205           |
|     | 0.5 mM calcium + 1 mM DBcAMP | 28.9 ± 2.2*          | 7,520 ± 620‡            | 4,590 ± 250           |
|     | 3.0 mM calcium | 4.1 ± 0.6            | 1,940 ± 110              | 1,190 ± 70            |
|     | 3.0 mM calcium + 1 mM DBcAMP | 8.6 ± 1.1§          | 2,680 ± 180∥            | 1,380 ± 120           |
| B.  | 0.5 mM calcium | 17.3 ± 0.8           | 5,210 ± 615              | 5,170 ± 110           |
|     | 0.5 mM calcium + 1 mM DBcAMP | 33.4 ± 2.1*          | 6,885 ± 880¶            | 5,100 ± 150           |
|     | 3.0 mM calcium | 5.0 ± 0.7            | 1,500 ± 90               | 1,350 ± 110           |
|     | 3.0 mM calcium + 1 mM DBcAMP | 9.1 ± 0.9§          | 2,710 ± 385∥            | 2,000 ± 485           |

Exp A: stored hormone was labeled with \[^{1}H\]lysine for 90 min at 3.0 mM calcium. The \[^{1}H\]lysine was chased by the addition of 250 \(\mu\)M unlabeled lysine and incubation was continued for an additional 60 min. Cells were harvested by gentle centrifugation, resuspended in media containing \[^{14}C\]leucine and the indicated calcium and dibutyryl cyclic AMP (DBcAMP) concentrations, and incubated for another 90-min period. Exp B: Performed as Exp A, except that \[^{1}H\]leucine and nonradioactive leucine were employed to initially label and chase the cells and \[^{14}C\]lysine was employed for the second labeling period. Results represent the average of three determinations ± the standard deviation.

* Differs from 0.5 mM Ca, \(P < 0.005\).
‡ Differs from 0.5 mM Ca, \(P < 0.01\).
§ Differs from 3.0 mM Ca, \(P < 0.005\).
¶ Differs from 3.0 mM Ca, \(P < 0.025\).
† Differs from 0.5 mM Ca, \(P < 0.05\).

TABLE III

Effect of \((1\)-Isoproterenol on the Secretion of Total and Radioactive Parathormone and Cellular Cyclic AMP

| Condition          | Cyclic AMP | Immunoactive hormone | Radiolabeled hormone |
|--------------------|------------|----------------------|----------------------|
|                    | fmol/10^6 cells | ng/10^6 cells | dpm | dpm/ng |
| Control            | 380 ± 50   | 15.9 ± 1.0          | 5,010 ± 330          | 315   |
| \(1 + \mu\)M (1)-Isoproterenol | 710 ± 160* | 29.4 ± 1.3‡         | 5,550 ± 670          | 187   |

The cells were incubated with 5 \(\mu\)Ci/ml \[^{14}C\]leucine for 90 min in the presence and absence of 1 \(\mu\)M (1)-isoproterenol. Immunoactive and radioactive parathormone content of the medium was determined as described in Materials and Methods. Companion incubations which did not receive labeled amino acid were terminated 15 min after the addition of (1)-isoproterenol for cellular cyclic AMP determinations. Results represent the average of six determinations ± standard deviation.

* Differs from control, \(P < 0.025\).
‡ Differs from control, \(P < 0.005\).

Agouge that has been reported to act through cyclic AMP in the parathyroid (4). Table III shows that the parathyroid cells incubated in the presence of (1)-isoproterenol released twice as much immunoactive parathormone to the medium and contained about twice the amount of cyclic AMP as did the controls. The bulk, if not all, of the increment in hormone was derived from the nonradioactive pool of hormone. Thus (1)-isoproterenol and dibutyryl cyclic AMP affected secretion of parathormone in the same manner.

A further link between hormone secretion and intracellular cyclic AMP levels in the parathyroid cells is portrayed in Fig. 4. This figure shows that the basal concentration of cyclic AMP in the cells varied inversely with calcium concentration of the incubation medium in general, reflecting the changes in parathormone secretion. Moreover, the changes in amount of cyclic AMP and the amounts of parathormone secreted elicited by calcium were in the range of those produced by the (1)-isoproterenol (compare Fig. 4 to Table III).

Examination of Fig. 2 reveals a major peak of radioactivity in the medium in addition to para-
thormone that we previously identified as secretory protein-I (also called parathyroid secretory protein) (24, 25). Since the secretion of this protein bears a close relationship to that of parathormone, we thought it of interest to compare its release to that of the hormone under our experimental conditions. Table IV lists the effect of calcium and dibutyryl cyclic AMP on the secretion of previously synthesized and more recently synthesized secretory protein-I. An antiserum for secretory protein-I was not available and hence the table does not include the actual amounts of this substance that was secreted. At 3.0 mM calcium, there was 90–95% less secretion of both newly and previously secretory protein compared to that at 0.5 mM calcium. This was a somewhat greater relative difference than for the secretion of parathormone (Table II) and is consistent with our earlier report (24). Dibutyryl cyclic AMP at both high and low calcium preferentially stimulated the secretion of previously synthesized secretory protein with little, if any, effect on secretion of newly synthesized protein (also see Fig. 2). The same results were obtained with (1)-isoproterenol in place of dibutyryl cyclic AMP (data not shown). Qualitatively, therefore, the actions of calcium, dibutyryl cyclic AMP, and (1)-isoproterenol on secretory protein-I and parathormone were similar.

**DISCUSSION**

In vitro radioisotopic studies suggest that complex relationships prevail in secretory tissues between preexisting secretory protein and that most recently synthesized by the tissue. Whether the preexisting or the newer peptide is favored for secretion seems to depend upon the tissue. In the parotid gland, Sharoni et al. (36) observed a preferential secretion of old granules over new. The stimulation of secretion by (1)-isoproterenol did not alter this preference but increased the rate of entry of newly made protein into the old granule pool. With pancreatic islets the bulk of the insulin released from the cells under stimulation by glucose or dibutyryl cyclic AMP was derived from preexisting stores, with perhaps a small component

![Figure 4](image-url)

**Figure 4** The secretion of parathormone and cellular cyclic AMP content in parathyroid cells as a function of medium calcium. Cells were incubated for 2 h at the indicated medium calcium concentrations. The cyclic AMP concentration (O) was determined in the cell pellet obtained by centrifugation while the secreted immunoreactive parathormone (●) was determined in the medium. Results represent the average of six determinations ± the standard deviation.

| Condition | ³H-‘old’ SP-I (dpm/10⁶ cells) | ³⁵C-new SP-I (dpm/10⁶ cells) |
|-----------|-------------------------------|------------------------------|
| 0.5 mM calcium | 17,280 ± 1,560              | 14,990 ± 1,270              |
| 0.5 mM calcium + 1 mM DBcAMP | 24,320 ± 1,290*             | 15,890 ± 1,310              |
| 3.0 mM calcium | 1,910 ± 240                  | 740 ± 100                   |
| 3.0 mM calcium + 1 mM DBcAMP | 2,670 ± 210†                 | 790 ± 90                    |

Conditions are as described for Table II, Exp A.

* Differs from 0.5 mM Ca, *P < 0.005.
† Differs from 3.0 mM Ca, *P < 0.025.

**TABLE IV**

Effect of Calcium and Dibutyryl Cyclic AMP on the Secretion of Previously Synthesized (‘Old’) and Newly Synthesized Secretory Protein-I (SP-I)
derived from preferentially secreted newer hormone (33, 34). The opposite situation seems to exist for the calcium-regulated release of parathormone by the parathyroid. MacGregor et al. (21), studying parathyroid slices, found that the specific radioactivity of the secreted hormone was always many-fold greater than that of the hormone in the storage pool of the tissue but approximated that of a deoxycholate-soluble fraction believed to contain the newly synthesized hormone. The stimulation of secretion achieved by lowering the calcium concentration of the incubation medium did not favor secretion of a greater proportion of older hormone. The contribution, if any, of the preexisting hormone pool to the total hormone release was not determined. MacGregor et al. (21) concluded that the newly formed hormone—presumably in prosecretory granules—"bypassed" the bulk of tissue stores during secretion.

Our present data are consistent with those of MacGregor et al. (21) and allow the further interpretations that both the preexisting and new pools of hormone participate in secretion by the tissue and that release from these two pools can be individually regulated. As was the case with parathyroid slices (21) the specific radioactivity of hormone in the incubation medium was substantially higher than that in the cells, in keeping with the preferential secretion of newly made hormone. We also found that with calcium as the modulator, the specific activity of the hormone released by the cells was the same at all levels of secretion (Table I). Since the secreted hormone was derived from both the older and more recently synthesized pools (Table II), we may conclude that calcium did not substantially alter the proportion of secreted hormone drawn from these pools, although the net amount secreted from each changed substantially. This result with dispersed cells differs somewhat from that of MacGregor et al. (21) with whole slices, in that they reported that at lower calcium concentrations somewhat more secreted hormone was derived from the more recently formed pool of hormone.

In contrast to the results with calcium, the stimulation of secretion by dibutyryl cyclic AMP or (1)-isoproterenol was accommodated by secretion almost exclusively from the pool of preexisting hormone. That this phenomenon was not a consequence of secretion by the cells at a rate greater than could be supplied by new synthesis can be deduced from the data of Table I: dibutyryl cyclic AMP affected release of nonradioactive hormone to the same degree regardless of the net secretory rate. We may also eliminate, as an explanation, changes in amount of available "new" hormone, since neither dibutyryl cyclic AMP nor (1)-isoproterenol affected the synthetic rate of proparathormone or the rate of degradation of intracellular parathormone.

Our results clearly point to the existence within the dispersed parathyroid cells of two slowly equilibrating and individually recruitable pools of parathormone. What is not clear is whether these pools coexist within single cells or whether, instead, they are contained within two physiologically different cell types. Studies in the literature do not allow one to choose with certainty between the two possibilities. Nakagami et al. (26) examined parathyroid gland function by electron microscopy autoradiography with [3H]tyrosine and [3H]galactose. They concluded that all of the cells of the gland were in about the same state of synthetic activity. Our autoradiographic examination of the dispersed cells (Fig. 1) agrees with their observation. Our results, therefore, could be explained by both parathormone pools existing in the same cell, providing the incorporation of radioactive label seen in the autoradiographic studies included as a component hormone synthesis. On the other hand, Roth and Capen (32) and Shannon and Roth (35) examined the ultrastructural features of the parathyroid of several species and have proposed that the cells of the gland pass through a discrete cycle of activity in which their synthetic and secretory activity varies broadly. This situation would readily fit the concept of two hormone pools residing in different parathyroid cells at different stages of activity. Additional studies will be required to decide between the above possibilities.

If the two hormone pools exist in the same cells, we may ask at what rate do the pools exchange? If the pools exist in different cells (that is, at different biosynthetic and secretory stages) we may ask at what rate do they cycle? In the data previously reported on parathyroid slices (20, 21), the entry of newly synthesized hormone into the pool of deoxycholate-resistant hormone (the "storage" pool) was so slow that no clear idea of equilibration time could be obtained. In the present data (Table II), the stimulation by dibutyryl cyclic AMP of hormone secretion from cells that had been prelabeled by a pulse of radioactive amino
acid produced about twice the increase in net hormone secretion as it did in radioactive hormone secretion. This nonequality suggests that the halftime of equilibration or, alternatively, of that required to pass through the synthetic to secretory stage is somewhat greater than 3–4 h.

Of interest are the mechanisms by which dibutyryl cyclic AMP and calcium affected the secretion of hormone from the tissue pools. Since the former agent acted primarily on the older hormone, it is conceivable that cyclic AMP acted directly or indirectly in the fusion of the secretory granule to the cell membrane during exocytosis. With calcium the situation could be more complex, since it enhances the degradation of newly formed parathormone (9, 17). This degradation alone could have limited the amount of new hormone that was secreted. Calcium could also have directly affected the passage of the new hormone (presumably in prosecretory granules [20, 21]) through the plasma membrane. In addition, since calcium directly affected the intracellular level of cyclic AMP (Fig. 4), it could also indirectly have influenced secretion from the preexisting granule pool.

The present results again show the similarity in secretion of parathormone and secretory protein-I. The fact that the latter protein was rapidly synthesized in the gland and that its secretion was under close regulation by calcium in the same direction as that of parathormone earlier suggested that it had an important function in the gland (24). In the present report, dibutyryl cyclic AMP preferentially enhanced the secretion of the preexisting pool of secretory protein-I over that of newly synthesized proteins, and calcium acted on both pools. Thus, secretory protein-I seems to be stored in the same manner as parathormone, and its secretion is modulated in a similar fashion. Although these and previous results do not provide a specific role for secretory protein-I, they strengthen the speculation that its secretion and that of the hormone are in some way coupled.

If the in vitro effects on secretion of parathormone by dibutyryl cyclic AMP and (1)-isoprote.-

enol observed in vitro are referable to events in vivo—and certain studies on stimulation of the gland in vivo (15, 23) suggest that they are—then we may conclude that the animal has within the parathyroid gland two different pools of parathormone that are subject to modulation by quite different physiological agents. The advantages to the animal in having such alternative secretory routes must yet be determined.

This research was supported in part by grant AM 18323 from the National Institutes of Health.

Received for publication 9 November 1978, and in revised form 29 January 1979.

REFERENCES

1. ABR, M. L., and L. M. SHELDWOOD. 1972. Regulation of parathyroid hormone secretion by adenylyl cyclase. Biochem. Biophys. Res. Commun. 48:396-401.
2. BROWN, E. M., R. J. CARROLL, and G. D. AUBRAH. 1977. Dopamine stimulation of cyclic AMP accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. Proc. Natl. Acad. Sci. U. S. A. 74:4210-4213.
3. BROWN, E. M., S. HURWITZ, and G. D. AUBRAH. 1976. Preparation of viable isolated bone parathyroid cells. Endocrinology. 99:1582-1588.
4. BROWN, E. M., S. HURWITZ, and G. D. AUBRAH. 1977. Beta-adrenergic stimulation of cyclic AMP content and parathyroid hormone release from isolated bovine parathyroid cells. Endocrinology. 100:1696-1702.
5. BROWN, E. M., S. HURWITZ, and G. D. AUBRAH. 1978. Beta-adrenergic inhibition of adenosine 3',5'-monophosphate accumulation and parathyroid hormone release from dispersed bovine parathyroid cells. Endocrinology. 3:893-899.
6. BROWN, E. M., S. HURWITZ, C. J. WOODWARD, and G. D. AUBRAH. 1977. Direct identification of beta-adrenergic receptors on isolated bovine parathyroid cells. Endocrinology. 100:1703-1709.
7. BUCKLE, R. M., A. D. CARE, C. W. COOPER, and H. J. GITELMAN. 1968. The influence of plasma magnesium concentration on parathyroid hormone secretion. J. Endocrinol. 42:529-534.
8. CHERROW, B. C., C. BAYLINE, J. WICHELSEID, M. SU, and A. W. NORMAN. 1975. Decrease in serum immunoreactive parathyroid hormone in rats and in parathyroid hormone secretion in vivo by 3,5-di-iodothyrocalcitonin. J. Clin. Invest. 56:607-687.
20. MacGregor, R. R., and D. V. Cohn. 1978. The intracellular pathway for parathormone synthesis and secretion. Clin. Orthop. Relat. Res. In press.

21. MacGregor, R. R., J. W. Hamilton, and D. V. Cohn. 1976. The bypass of tissue hormone stores during the secretion of newly synthesized parathyroid hormone. Endocrinology. 97:178-187.

22. Matsumoto, S., and I. E. Dumont. 1972. Effect of calcium on horse parathyroid gland adenylate cyclase. Biochim. Biophys. Acta. 284:227-234.

23. Metz, S. A., J. L. Beato, D. J. Baylink, and P. R. Rottman. 1978. Neuroendocrine modulation of calcium and parathyroid hormone in man. J. Clin. Endocrinol. Metab. 47:1476-1485.

24. Morrissey, J. J., and D. V. Cohn. 1978. The effects of calcium and magnesium on the secretion of parathormone and parathyroid secretory protein by isolated porcine parathyroid cells. Endocrinology. 103:2081-2090.

25. Morrissey, J. J., J. W. Hamilton, and D. V. Cohn. 1978. Secretion of parathormone and glycosylated proteins by parathyroid cells in culture. Biochim. Biophys. Acta. 821:127-1286.

26. Nakagami, K., H. Warshavsky, and C. P. LeBlond. 1971. The elaboration of protein and carbohydrate by rat parathyroid cells as revealed by electron microscope radioautography. J. Cell Biol. 51:596-607.

27. Oldham, J. B., J. A. Fisher, C. C. Capen, G. W. Szemere, and C. D. Arnaud. 1971. Dynamics of parathyroid hormone secretion in vivo. Am. J. Med. 50:350-357.

28. Pate, H. M., and A. B. Luckhardt. 1942. Relationship of a low blood calcium to parathyroid secretion. Endocrinology. 31:384-392.

29. Pelc, S. R. 1956. The stripping film technique of autoradiography. Int. J. Appl. Radiat. Isot. 1:172-177.

30. Reisfeld, R. A., U. J. Lewis, and D. E. Williams. 1962. Disc electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature (Lond.). 195:283.

31. Rodríguez, H. J., A. Morrison, E. Slatopolsky, and S. Klaine. 1978. Adenyl cyclase of human parathyroid gland. J. Clin. Endocrinol. Metab. 47:319-325.

32. Roth, S. L., and C. C. Capen. 1974. Ultrastructural and functional correlations of the parathyroid gland. Int. Rev. Exp. Pathol. 13:161-231.

33. Sandos, H. J., W. J. Boldt, and D. F. Steiner, 1972. Studies on the secretion of newly synthesized proninulin and insulin from isolated rat islets of Langerhans. J. Clin. Invest. 51:1476-1485.

34. Sandos, H., and G. M. Grosvenor. 1973. Dynamic synthesis and release of insulin and proninulin from perfused islets. Diabetologia. 21:354-360.

35. Shand, A. A., Jr., and S. I. Roth. 1974. An ultrastructural study of acid phosphatase activity in normal, adenomatous and hyperplastic (chief cell type) human parathyroid glands. Am. J. Pathol. 174:943-952.

36. Skalski, T. S., and M. Schramm. 1977. Secretion of old versus new exportable protein in rat parotid slices. J. Cell Biol. 78:107-122.

37. Sherwood, L. M., J. Herman, and C. A. Basili. 1970. Parathyroid hormone secretion in vivo. Regulation by calcium and magnesium ions. Nature (Lond.). 225:1056-1058.

38. Sherwood, L. M., G. P. Mayer, C. R. Ramberg, D. S. Kranfeld, G. D. Aurbach, and J. T. Potit, Jr. 1968. Regulation of parathyroid hormone secretion. Proportional control by calcium, lack of effect of phosphate. Endocrinology. 83:1043-1051.

39. Williams, G. A., G. K. Hargis, E. N. Bowser, W. J. Henderson, and N. J. Martinez. 1973. Evidence for a role of adenine 3',5'-monophosphate in parathyroid hormone release. Endocrinology. 92:687-691.