ENERGY-DEPENDENT INTRACELLULAR TRANSLOCATION OF PROPARATHORMONE

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ABSTRACT

We previously suggested that after synthesis, proparathormone is transferred from rough endoplasmic reticulum to the Golgi region where its conversion to parathormone occurs. We have attempted to define more closely this transfer process. In the first type of study, bovine parathyroid slices were incubated with [^{3}H]leucine for 10 min and then radioisotope labeling was restricted by addition of a large excess of nonradioactive leucine. Under these conditions, more than 90% of the initially labeled proparathormone was converted to parathormone in 40 min. Lowered temperature in the chase period markedly inhibited the conversion. Several chemical agents were employed individually in the chase period to examine their effect on the conversion process. Antimycin A, dinitrophenol, oligomycin, and anaerobiosis (N_2) inhibited the conversion, whereas sodium fluoride and cycloheximide had no effect. In the second type of study, parathyroid slices were incubated with [^{3}H]leucine for the entire incubation period. Lowered temperature and inhibitors of energy metabolism and microtubular function all lengthened the interval (lag) between the initial synthesis of [^{3}H]proparathormone and the first appearance of [^{3}H]parathormone. Cycloheximide, Tris, and chloroquine decreased the rates of protein synthesis and conversion, respectively, but none had any effect on the lag. We interpret the lag to represent the time of transit for proparathormone from rough endoplasmic reticulum to the Golgi region. We conclude that this transfer process is independent of the synthesis of the prohormone and its conversion to the hormone. Moreover, this translocation requires metabolic energy and appears to be mediated by microtubules.

The parathyroid gland is of interest in terms of its specific role in forming and secreting parathormone as well as in its serving as a model for the study of the genesis, transfer, and processing of exportable peptides. Based on a variety of studies, there has developed general acceptance of the hypothesis that exportable peptides (for example, immunoglobulins, albumin, pancreatic enzymes, and insulin) are synthesized on membrane-bound ribosomes as larger precursors (1, 8, 10, 14, 26, 27, 29, 31). It also has been proposed that a peptide sequence on the amino-terminal end of the nascent chain serves as a "signal" for passage of the chain into the cisternal space of the endoplasmic reticulum (2, 10). Once inside, the "signal" sequence is removed, and the residual peptide is transferred to the Golgi zone where packaging into storage vesicles commences before secre-

tion. During this process, the peptides may undergo further processing, including proteolytic cleavage (30), hydroxylation (1), and glycosylation (20).

Nakagami et al. (22), employing electron microscope autoradiography, provided direct evidence that this mode of processing applied to the parathyroid gland. Their data indicated that newly synthesized peptides moved from rough endoplasmic reticulum to the Golgi zone and then to secretory granules (22). Our biochemical studies on proparathormone biosynthesis and parathormone formation are also in accord with this scheme. Our data (6) led us to suggest that the prohormone, after synthesis on the rough endoplasmic reticulum, is transferred to the Golgi zone where proteolytic cleavage to the hormone commences. In the present report we have examined the kinetics of the transport process itself by measuring the effects of temperature and various inhibitors on the time lag (interpreted to be transit time) between synthesis of the prohormone and the appearance of the hormone. We find that the transport process requires metabolic energy, is independent of the synthesis and conversion of the prohormone, and involves the participation of microtubules.

MATERIALS AND METHODS

Preparation and Incubation of Tissue Slices with ^{3}H-Labeled Amino Acids

This procedure was performed as described earlier (5). In pulse-chase studies, 30–50 mg slices were incubated with 2–10 μCi of [^{3}H]leucine or [^{3}H]lysine in 0.25 ml of Earle's buffer for 10 min. The slices were then transferred to fresh buffer containing 2 mM of nonradioactive leucine or lysine and other agents as specified and incubation was continued for up to 40 min more. In the time-course studies, the tissue was incubated with the radioactive amino acids for the entire period of incubation. Reaction was terminated by chilling the samples in an ice bath followed by addition of 2 ml of a solution containing 8 M urea, 0.2 N HCl, and 0.1 M cysteine.

Isolation of Radioactive Hormonal Peptides from Tissue

Radioactive proparathormone and parathormone were isolated from the tissue and incubation medium as described earlier (4). To achieve more quantitative recovery of the peptides, 6 M urea was included in the salt gradients during carboxymethylcellulose chromatography. This yielded about 95% recovery of both parathormone and proparathormone. Therefore the use of radioactive proparathormone and parathormone as an internal standard (5) was dispensed with in certain experiments.

Incorporation of radioactive amino acids into total proteins was measured in the hot TCA-insoluble material from the initial tissue homogenate.

In Vitro Conversion of Proparathormone to Parathormone

A particulate fraction that was sedimented at 190,000-*g* by centrifugal force from a post-20,000-*g* supernate of a bovine parathyroid gland homogenate was prepared as described elsewhere (19). It was resuspended in 0.1 M sodium phosphate, pH 7.4, at a concentration of 100 μg protein/ml. [^{3}H]Proparathormone was incubated with this suspension for 30–60 min in the presence or absence of the testing agents at the indicated temperatures. At the end of the incubation, the mixture was chilled, diluted with 6 M urea-0.05 M ammonium acetate, pH 5.3, applied to a carboxymethylcellulose column as described earlier (5), and [^{3}H]parathormone product and unreacted [^{3}H]prohormone were eluted.

Measurement of Tissue Respiration

Oxygen consumption by tissue slices was measured by a Gilson respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.). Tissue was incubated in Krebs-Ringer phosphate buffer in a single sidearm Warburg flask (center well, KOH) for periods of up to 120 min until the rate of oxygen consumption became constant. Test agents were added from the sidearm, and the incubation was continued for another 90 min. Readings were taken every 15–20 min.

Radioactive Assay

All radioactive samples were assayed by dissolving them in a toluene-Triton based scintillation fluid and counting in a Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Internal standards were employed for determination of efficiency.

Materials

Radioactive amino acids were purchased from New England Nuclear Corp. (Boston, Mass.). Tris, antimycin A, dinitrophenol, oligomycin, sodium fluoride, and rotenone were obtained from Sigma Chemical Co. (St. Louis, Mo.); colchicine and vinblastin from Calbiochem (San Diego, Calif.); cytochalasin B from Aldrich Chemical Co., Inc. (Milwaukee, Wis.); and D_2O from Bio-Rad Laboratories (Richmond, Calif.). All other chemicals were reagent grade and were obtained from various suppliers.

RESULTS

After 10 min of incubation of parathyroid slices with [^{3}H]leucine at 37°C, proparathormone con-

tained substantial radioactivity, whereas the parathormone pool contained almost none (Fig. 1). When the incorporation of [^{3}H]leucine was limited at this point by addition of a large excess of nonradioactive leucine and incubation was continued (chase period), radioactive proparathormone disappeared and there was a concomitant appearance of radioactive parathormone. After 40 min of chase at 37°C, the majority of radioactive prohormone was converted to hormone. When the chase incubations were conducted at lower temperatures, the rates of conversion of prohormone to hormone decreased. Thus at 31, 25, and 20°C, the amounts of hormone formed after 40 min were 62, 26, and 4%, respectively, of that formed at 37°C.

We also observed a negative effect of temperature when we assayed the capacity of a particulate fraction prepared from parathyroid tissue to convert the prohormone to the hormone. This particulate fraction is believed to contain the physiological proparathormone-parathormone convertase activity (19). At 31, 25, and 20°C, the conversion by the in vitro enzyme preparation was 71, 40, and 20%, respectively, of that at 37°C. The fact that the effect of temperature on conversion was less with the particulate enzyme preparation than with the intact tissue slices suggested that a second temperature-sensitive event was operative in the slices.

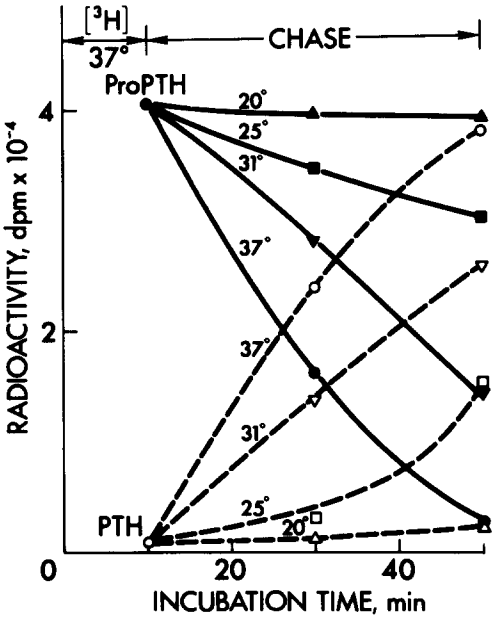

FIGURE 1 Effect of incubation temperature on proparathormone conversion during chase period. Fresh bovine parathyroid slices were incubated with [^{3}H]leucine for 10 min and chased with nonradioactive leucine as described in the text. Radioactive-labeled proparathormone and parathormone were isolated from the tissue according to the modified standard method described in Materials and Methods. Closed symbols represent proparathormone (ProPTH); open symbols, parathormone (PTH). Each point represents the average value of duplicate samples.

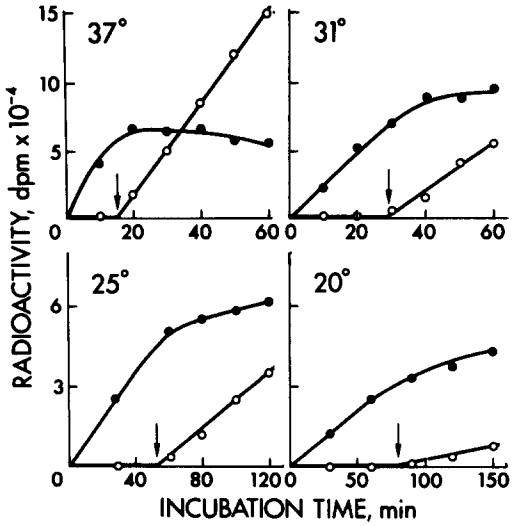

FIGURE 2 Time-course of [^{3}H]leucine incorporation into hormonal peptides at different temperatures. Fresh bovine parathyroid slices were incubated with [^{3}H]leucine for the indicated length of time. Each point represents the average value of duplicate samples. Closed symbols represent proparathormone; open symbols, parathormone. The small arrow in each panel indicates the extrapolated time at which [^{3}H]parathormone first appears.

We therefore studied the effect of temperature on the biosynthesis of proparathormone and the formation of parathormone by the slices in greater detail. Fig. 2 shows the results of a series of time-course studies performed at different temperatures. These experiments differed from those shown in Fig. 1 in that the temperature was not changed for each incubation and no chase period was employed. The lower the temperature the lower were the rates of formation of both the prohormone and hormone. A similar decrease in the rate of total protein synthesis also occurred (data not shown). Of major interest in these kinetics was the substantial lengthening of interval (lag) between synthesis of the prohormone and onset of formation of the hormone: namely, 15 min at

37°C; 30 min at 31°C; 55 min at 25°C; and 80 min at 20°C (arrows, Fig. 2). Since the lag period had earlier been interpreted to represent the time required for the prohormone to reach its site of conversion (7), these results suggested that lowering of temperature affected the synthesis of the prohormone, its transfer to its site of conversion, and its enzymic conversion to the hormone.

Since it seemed reasonable that the inhibitory effects noted at lowered temperatures were secondary to a reduction in metabolism of the tissue, we tested several metabolic inhibitors at 37°C for their effects on conversion and on length of lag period. Table I shows that in pulse-chase experiments antimycin A and dinitrophenol each decreased the amount of [^{3}H]parathormone formed in a dose-dependent manner with greater than a 90% inhibition at a concentration of 10^{-4} M. Oligomycin (5×10^{-5} M), rotenone (10^{-4} M), and anaerobiosis (N_2) each inhibited between 40 and 50%. NaF (2×10^{-3} M), 2-deoxy-D-glucose (5×10^{-3} M), and cycloheximide (4×10^{-4} M) had little effect. In each individual experiment the sum of radioactive parathormone and proparathormone in the treated tissue was the same as that in the control. Therefore, we could attribute the decreases in amount of parathormone directly to inhibition of the conversion step rather than to alteration in the possible degradation of either the hormone or prohormone. In separate experiments we found that antimycin A (10^{-4} M) inhibited tissue respiration by 90%, whereas dinitrophenol (10^{-4} M) and NaF (2×10^{-3} M) had no effect. Two of the typical inhibitors were also tested directly on the particulate-associated convertase. Dinitrophenol (10^{-4} M) did not affect the conversion activity, and antimycin A (10^{-4} M) inhibited only about 50% (Table II). These results there-

TABLE I

Effect of Metabolic Inhibitors on Conversion of ProPTH to PTH in Slices during Chase Period*

Condition		PTH formed, *dpm* mean ± SE	(*n*)	Inhibition
				%
Before chase (initial):		897 ± 151	(30)	–
After chase:				
Control	–	26,360 ± 360	(34)	–
Antimycin A	1×10^{-6} M	16,994 ± 800	(7)	37
	1×10^{-5} M	14,750 ± 700	(16)	46
	5×10^{-5} M	6,650 ± 420	(8)	77
	1×10^{-4} M	3,190 ± 570	(10)	91
Dinitrophenol	1×10^{-6} M	18,560 ± 970	(5)	31
	1×10^{-5} M	16,370 ± 1,390	(14)	39
	5×10^{-5} M	8,660 ± 1,370	(6)	70
	1×10^{-4} M	2,950 ± 490	(6)	92
Oligomycin	5×10^{-5} M	15,920 ± 1,440	(2)	41
Rotenone	1×10^{-4} M	13,430 ± 1,060	(4)	51
N_2	–	15,800 ± 2,690	(7)	41
NaF	2×10^{-3} M	25,130 ± 1,250	(7)	5
Cycloheximide	4×10^{-4} M	26,400 ± 500	(4)	0
2-Deoxy-D-glucose	5×10^{-3} M	26,000 ± 900	(4)	1

In each experiment, six samples were pulsed for 10 min with radioactive leucine (pulse period). Duplicate samples were taken at this time for measuring incorporation into proparathormone and parathormone during the pulse (initial sample). Two samples were chased for 40 min with nonradioactive leucine in a fresh medium that did not contain [^{3}H]leucine (control sample). Two samples were chased as control samples with the addition of the testing agent (experimental sample). The sum of labeled ProPTH and PTH was the same for initial, control, and experimental samples. Since the amount of tissue and isotope varied somewhat in different batches, which resulted in different total incorporation, we normalized the sum of prohormone and hormone to one typical value, 30,600 dpm. The table lists the normalized value of PTH for each incubation (n = number of incubations). Percent inhibition = 100 × (Net PTH formed during chase by test sample/Net PTH formed during chase by control sample).

* The following abbreviations are used in Tables I and II: ProPTH, proparathormone; PTH, parathormone.

TABLE II

Effects of Inhibitors on Conversion of ProPTH to PTH by Particulate-Associated Convertase

Condition	^{3}H-PTH formed	Control
	dpm	%
Control	2,870	–
Antimycin A, 10^{-4} M	1,370	48
Dinitrophenol, 10^{-4} M	2,800	98
Colchicine, 10^{-3} M	2,860	100
Vinblastin, 10^{-4} M	3,070	107

15,000 dpm of ^{3}H-ProPTH were incubated for 60 min with a particulate fraction obtained from bovine parathyroid homogenate. Data are averages of duplicate samples that varied no more than 10% from each other.

fore indicated that energy, specifically that derived from oxidative phosphorylation, is required to facilitate the overall conversion of proparathormone to parathormone. Fig. 3 shows the effect of antimycin A on the time-course of amino acid incorporation into prohormone and hormone when the inhibitor was present during the entire incubation. Dinitrophenol was similarly tested (data not shown). Under these conditions both agents increased the lag to about 26 min from the control value of 16 min. In addition, both inhibited the rate of the prohormone synthesis and the rate of hormone formation.

Since the rate of prohormone synthesis was reduced by the metabolic inhibitors (Fig. 3) as well as by the lowered temperatures (Fig. 2), it was possible that the lengthening of the lag was secondary to this reduced rate of synthesis. For this reason, we lowered the rate of prohormone synthesis with cycloheximide and examined the effect of this treatment on the length of the lag. Fig. 4 shows that at 0.05 μg/ml the synthesis of proparathormone was inhibited by about half, a level similar to that brought about by lowered temperatures or by the metabolic inhibitors. This treatment did not alter the normal 15-min lag, thus indicating that the length of lag was independent of the rate of prohormone synthesis.

Earlier we reported that two groups of agents will specifically inhibit the conversion process (5, 19). The first group consists of nonamphoteric organic amines that appear to work through disruption of the Golgi apparatus (5). The second group consists of inhibitors of proteolysis which presumably act directly on the convertase system (19). Fig. 5 shows that the amine inhibitor, Tris, decreased the rate of parathormone formation but did not affect the lag. A second amine, diethylamine, was also tested and yielded a similar result (data not shown). Likewise, chloroquine, an inhibitor of the particulate-associated convertase (19), did not lengthen the lag (Fig. 6) even though it decreased the rate of parathormone formation. The gradual rise in the rate of hormone formation may represent a recovery of the slices from the chloroquine poisoning. In these studies, typified by that shown in Fig. 5, there was an apparent increase in the amount of proparathormone that was formed in the presence of the amine inhibitors and chloroquine. This appears to have been the result of the diminution in conversion of the prohormone molecule – not from an increase in synthetic rate.

Finally, the inhibitors of microtubule function, colchicine, vinblastin, and D_2O, and the microfilament inhibitor, cytochalasin B, were tested for their effect on the length of lag. Fig. 7 shows a typical result of one of three identical experiments with colchicine. The length of lag increased from the control value of 18 min to 25 min. Vinblastin (Fig. 8) and D_2O (Fig. 9) also increased the length of lag. Cytochalasin B affected neither the conversion process nor the lag (data not shown). In a

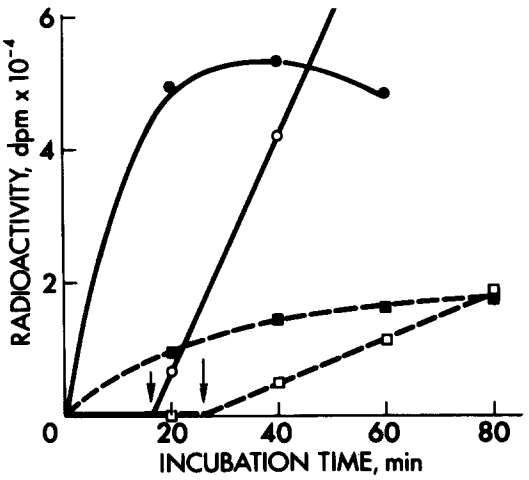

FIGURE 3 Effect of antimycin A on time-course of [^{3}H]lysine incorporation into hormonal peptides. Fresh bovine parathyroid slices were incubated with [^{3}H]lysine in the absence (control) or presence of antimycin A (1×10^{-5} M). The continuous line and circles represent the values of control samples. The dashed line and squares represent the value of test sample. Closed symbols represent proparathormone; open symbols, parathormone. The small arrows indicate the extrapolated time at which [^{3}H]parathormone first appears.

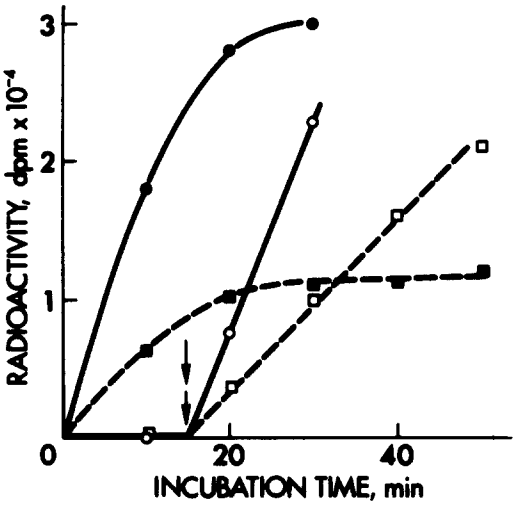

FIGURE 4 Effect of cycloheximide on time-course of [³H]leucine incorporation into hormonal peptides. Fresh parathyroid slices were incubated with [³H]leucine in the absence (control) or presence of cycloheximide (0.05 μg/ml). See Fig. 3 for other details.

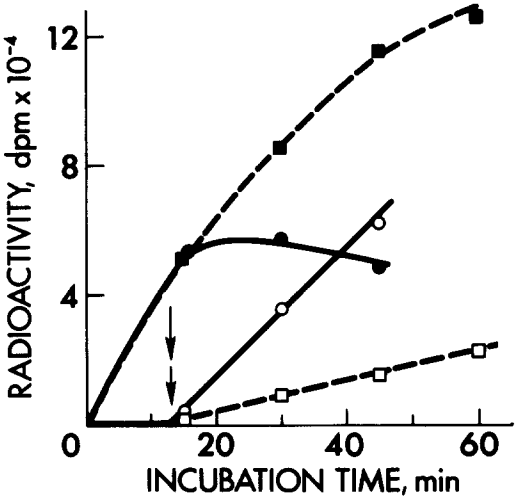

FIGURE 5 Effect of Tris on time-course of [³H]lysine incorporation into hormonal peptides. Fresh bovine parathyroid slices were incubated in the absence (control) or presence of Tris (20 mM). 45 min later, [³H]lysine was added to each incubation tube. Incubation was terminated 15, 30, 45, and 60 min after the addition of [³H]lysine. See Fig. 3 for other details.

separate study, vinblastin and colchicine were tested for the effect on the conversion in vitro of proparathormone to parathormone by a particulate-associated converting activity (19). Table II shows that these agents had no effect.

In summary, lowered incubation temperature, inhibitors of energy metabolism, and inhibitors of microtubular function all increased the length of the lag. The inhibitor of protein synthesis, inhibi-

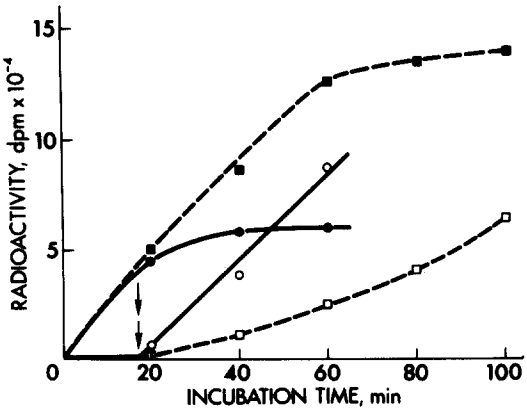

FIGURE 6 Effect of chloroquine on time-course of [³H]leucine incorporation into hormonal peptides. Fresh bovine parathyroid slices were incubated in the absence (control) or presence of chloroquine (5×10^{-4} M). 30 min later, [³H]leucine was added to each incubation tube. Incubation was terminated at the indicated time after addition of [³H]leucine. See Fig. 3 for other details.

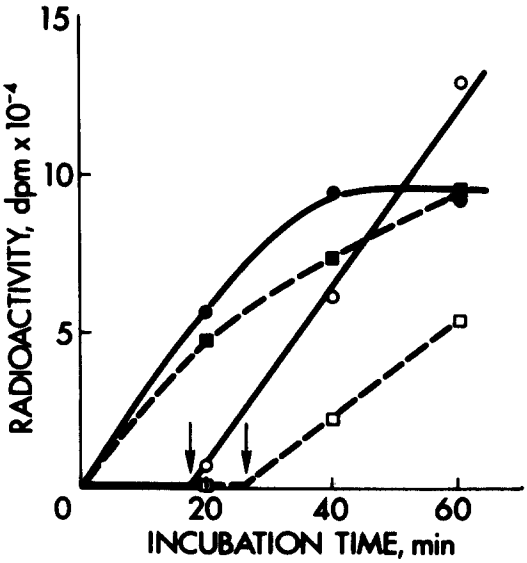

FIGURE 7 Effect of colchicine on time-course of [³H]lysine incorporation into hormonal peptides. Fresh slices were incubated in the absence (control) or presence of colchicine (5×10^{-4} M). After 60 min, [³H]lysine was added to each incubation tube. The incubation was terminated at the indicated time after addition of [³H]lysine. See Fig. 3 for other details.

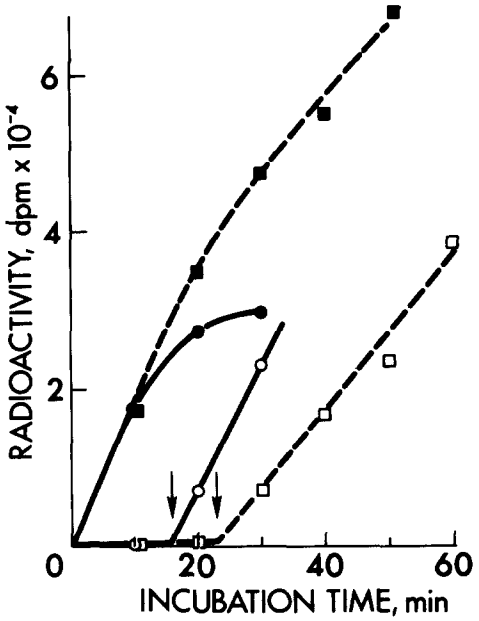

FIGURE 8 Effect of vinblastin on time-course of [³H]leucine incorporation into hormonal peptides. Fresh slices were incubated with or without vinblastin (1×10^{-4} M). After 60 min, [³H]leucine was added to each incubation tube. The incubation was terminated at the indicated time after addition of [³H]leucine. See Fig. 3 for other details.

tors of the particulate-associated prohormone-hormone convertase, and the microfilament inhibitor had no effect on the lag.

DISCUSSION

The present data show that the lag between synthesis of proparathormone and formation of parathormone is tied closely to energy metabolism of the tissue and is independent of the rate of synthesis of the prohormone and the rate of conversion of the prohormone to the hormone. Thus dinitrophenol, an uncoupler of oxidative phosphorylation, antimycin A, an inhibitor of electron transport, and lowered temperatures of incubation all substantially lengthened the lag. But when synthesis of total protein including the prohormone was inhibited with cycloheximide or when formation of parathormone was blocked by chloroquine, an inhibitor of proteolysis, and by Tris and diethylamine, amines which affect Golgi structure (5), the length of the lag did not change.

On the basis of the time required for translocation of exportable proteins from rough endoplasmic reticulum to Golgi apparatus (12, 22, 24), we earlier postulated that the delay in formation of parathormone represents the time required for transit of proparathormone from its site of synthesis to its site of conversion (6, 7). In the present study we have continued to view our results in this light since our data are fully consistent with this interpretation. However, there are at least two alternative interpretations of the physical meaning of the lag period that bear consideration. The first is that the lag represents the time required for accumulation of sufficient prohormone at or near its site of synthesis for proteolytic conversion to commence. This would be analogous to the well-documented cooperative effects in enzyme systems in vitro in which the catalytic rate of an enzymatic reaction is enhanced as the amount of substrate is increased. Such cooperative effects, however, do not appear to be a viable explanation of our results since the tissue was synthesizing prohormone before exposure to radioactive amino acid; hence the amount of prohormone would not be expected to change after introduction of ra-

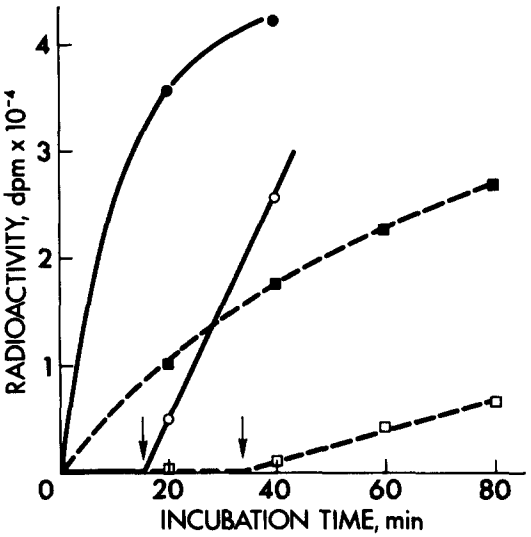

FIGURE 9 Effect of D_2O on time-course of [³H]leucine incorporation into hormonal peptides. In this experiment, the testing solution was Hanks' salt solution made with H_2O (control) or D_2O. After a standard preincubation of 60 min in the regular Hanks' solution, the fresh slices were incubated in regular Hanks' solution (control) or D_2O-Hanks' solution for another 60 min. Afterwards, tissues were transferred to fresh regular Hanks' solution (control) or fresh D_2O-Hanks' solution, each containing [³H]leucine. Incubation was terminated at the indicated time after exposure of tissues to [³H]leucine. See Fig. 3 for other details.

dioisotopic label. A second alternative interpretation is that the lag represents a composite measure of both the time required for accumulation of a specific amount of prohormone to enable translocation to proceed and the time required for actual transit. In this view, one must postulate that translocation of peptide occurs in quanta—not dissimilar to the movement of secretory proteins contained in vesicles (granules) from Golgi apparatus to cell membrane. This interpretation is not consistent with our data. Specifically, cycloheximide, which decreased the rate of prohormone synthesis by at least 50% (Fig. 4), did not lengthen the lag. At present, therefore, the original interpretation that lag represents actual transit time still remains the best explanation of the phenomenon.[1]

Accordingly, our data indicate that the physical movement of the prohormone to its site of conversion requires metabolic energy. This need for energy would seem to be a general one since a similar requirement has been reported in the cases of intracellular transfer of proteins in the nerve cell (23), of pancreatic exocrine proteins (13), of proinsulin (28), and of procollagen (16). Our data further indicate that in the parathyroid, at least, the transfer mechanism is independent of the rate of synthesis and the subsequent proteolytic modifications (conversion) of the prohormone.

The specific nature of this intracellular transport mechanism remains to be elucidated. It has been suggested that exportable proteins move freely within a permanent cisternal connection from endoplasmic reticulum to the Golgi apparatus (21) or, alternatively, that they are carried between these two sites within discrete vesicles (12, 24). In either case, the newly synthesized peptide would be restricted to particulate structures—a condition shown to exist in the parathyroid gland for proparathormone (17). Our finding that colchicine, vinblastin, and D_2O lengthen the transit time of the prohormone amplifies the earlier report of Kemper et al. (15) and suggests that a functional microtubular system is required for this transport. Indeed the presence of microtubules in the parathyroid and the ability of colchicine to deplete their number have recently been observed (25). Presumably, if the microtubular elements act to translocate the prohormone within the cisternal space of the endoplasmic reticulum, then the requirement for cellular metabolism would be related to supplying the energy necessary for this transfer. On the other hand, caution must be exerted in interpreting these data since these agents, particularly at high concentration, might not be specific for the disruption of microtubules and could be acting via other cellular mechanisms such as direct inhibition of energy metabolism.

Since microtubules are disaggregated at low temperature (11), one might attribute the extended "transit time" at low temperature (Fig. 2) to a direct physical effect on microtubules rather than to reduction in the supply of metabolic energy. While this possibility has not been ruled out in the present study, disaggregation of microtubular structure has only been observed at temperatures substantially lower than those tested in the present work—usually about 4°C. Consequently, we think that the effects of lowered temperature reported herein relate primarily, although not necessarily solely, to restriction in energy metabolism.

With these considerations in mind and based on several earlier studies, the following pattern for the processing of parathormone emerges. The precursor of parathormone is first synthesized as preproparathormone (14)—a molecule that contains a peptide segment on the amino-terminal end preceding the proparathormone structure. In accord with the recently advanced "signal" hypothesis of Blobel and Dobberstein (2), this peptide segment may serve as a signal for entry of the nascent preproparathormone chain from its ribosomal site of synthesis into the cisternal space. Immediately upon entry, this peptide portion is cleaved, yielding proparathormone—the immediate precursor of parathormone (8). The prohormone is then transferred to the Golgi region by an energy-dependent, microtubule-mediated process. At this site proteolytic conversion to parathormone occurs (5). From this point the hormone may undergo secretion subsequent to packaging into secretory vesicles or may be secreted without prior equilibration with the hormone in the pool of secretory granules (17).

Although the intracellular movement of parathyroid peptides specifically, and other exportable

[1] A direct measure of prohormone and hormone in isolated subcellular organelles of parathyroid gland would be useful in strengthening this conclusion. At present, however, a satisfactory separation of smooth and rough endoplasmic reticulum and Golgi apparatus from bovine parathyroids has not been achieved, owing in part to the difficulty with which bovine glands are dispersed. Because of the high fat content and copious connective tissue, it is necessary to subject the tissue to high shearing forces in order to disrupt it—a procedure which apparently disorders the integrity of the subcellular organelles.

peptides in general, might be unique, a strikingly similar movement of protein molecules on the surface of cell membranes has been described. This is the phenomenon of capping (32). In this process, when proteins of the plasma membrane are bound together by multivalent antibodies, they move unidirectionally to one pole of the cell. This movement, like intracellular translocation, is energy-dependent and is blocked by inhibitors of microtubular function (9, 32). It is conceivable that vectorial movement of peptides both inside the cell and on its surface is part of a coupled flow of cellular constituents during general physiological functioning. A proposal encompassing such a combined movement to account for capping has recently been advanced by Bretscher (3).

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