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COMMON MECHANISMS OF HORMONE SECRETION

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INTRODUCTION

With the exception of the thyroid gland, which stores hormones extracellularly in the lumen of its follicles, the endocrine tissues store their hormones intracellularly (1). Two main forms of intracellular storage must be recognized: 1. The steroid-secreting glands which store very little amounts of hormone but large quantities of precursor. The adrenal cortex, the ovary, and the testis all belong to this category of endocrine glands in which the presence of secretory granules has not been demonstrated. However, these tissues store large amounts of esterified cholesterol in lipid droplets, and this represents a depot of hormone precursor (1, 2). 2. Endocrine tissues which store large quantities of hormones in membrane-bound organelles, the secretory granules. A similar way of storing secretory products may be observed in exocrine glands (3, 4) and also in nervous tissues which synthesize and release novel transmitter substances (5, 6).

The main concern of this review is with the secretory events which occur in those cells storing hormones in subcellular granules. Among this type of cell are those of the adenohypophysis, the islets of Langerhans of the pancreas, the adrenal medulla, the parathyroid gland, the parafollicular cells of the thyroid, and the neurosecretory cells of the hypothalamus-neurohypophysial system. Because these tissues share the common feature of storing their hormones in granules, it is possible that the cellular and molecular mechanisms of secretion are also similar. Therefore, this article seeks to unify published observations concerning the events involved in the secretory process of these tissues. Because of space limitations, it has been necessary to make an arbitrary selection of references to discuss and illustrate the ideas presented here; in many cases there are other references of equal value which would also support these ideas.

STORAGE OF HORMONES IN GRANULES

Evidence for the storage of hormones in subcellular particles has come to light from results obtained through morphological and biochemical techniques. Centrifugation studies have demonstrated both that the hormone activity in the homogenates of endocrine glands can be sedimented and that the hormone-containing particles of these sediments can be further separated from other subcellular particles by density-gradient centrifugation techniques (1, 7–10). Electron microscopic analysis of the subcellular fractions containing the hormone activity has revealed the presence of membrane-bound granules similar to those observed in intact tissues (7–10). Granule preparations of a quite different degree of purity have been isolated from different encodrine tissues (7–11). Chromaffin granule fractions of a high degree of purity have been isolated from the adrenal medulla (7, 12, 13). The subsequent chemical analysis of these granules (14) has provided a large and valuable body of information on the mechanisms of storage (14, 15) and release of adrenal medullary hormones (14). Therefore it is important that efforts be made to obtain granule preparations of a high degree of purity from other endocrine tissues.

In order to assess the importance of storage granules it is necessary to point out some of the advantages of this type of hormone storage: (*a*) the hormones may reach very high concentrations within the granules (that is, up to 0.5 M in chromaffin granules); consequently the capacity for hormone storage of these tissues is very high; (*b*) the hormone is protected from enzyme inactivation or catabolism (that is, monoamine oxidase in the adrenal medulla); (*c*) since the granules are of approximately the same volume in any given tissue, each granule provides a fixed amount of hormone (quantum) to be released; (*d*) the granules facilitate the intracellular transport of large quantities of hormones, especially in the case of the granules of the neurohypophysis which contain vasopressin and oxytocin, although it is likely that some kind of mechanism exists in all secretory tissues for the transport of granules from the Golgi apparatus to the cell periphery; and (*e*) by fusing with the plasma membrane during the release reaction (see below), the granules thus expose their contents directly to the cell exterior.

THE TRANSMEMBRANE POTENTIAL IN SECRETORY CELLS

The transmembrane potentials of the secretory cells are generally much lower than those of the muscle and nerve cells. The published values of resting membrane potentials of the secretory cells under discussion here ranged from –12 to –41 mV (16, 17). The origin of the potential in secretory cells seems to be the same as that in excitable cells, since the intracellular concentration of Na^+ and K^+ are similar to those in nerve and muscle (16, 17). However, secretory cells have higher ratios of membrane permeability of Na^+ to K^+ ($P_{Na}+/P_K+$) than nerve and muscle (16), which would explain the low resting potential observed in secretory cells. The high $P_{Na}+/P_K+$ ratio, together with the large area to volume ratio of secretory cells, is probably responsible for the rapid change in the intracellular concentrations of

ions produced in these cells during stimulation (16, 17). Stimulation by raising the extracellular concentration of K^+ depolarizes the endocrine cells, but the potential values always remain negative (18–21). Furthermore, secretory cells have gap junctions which are membrane areas of low resistance (17). For this reason, comparison of membrane resistance in different kinds of secretory cells would be unjustified (17).

IONIC REQUIREMENTS FOR RELEASE

Role of Divalent Cations

In 1928 Houssay & Molinelli first suggested the importance of Ca^{2+} in hormone secretion (22). However, it was W.W. Douglas who conceived of the role of Ca^{2+} in the secretory process as a general one, and who gave the name of *stimulus-secretion coupling* to the events involved in the secretory process because of the similarity to the excitation-contraction coupling in muscle (23). Douglas and co-workers not only established the role of Ca^{2+} in the secretory process of the adrenal medulla (24) but they also extended these observations to the neurosecretory cells of the posterior pituitary (25) and to the submaxillary gland (26). Later on, the requirement of Ca^{2+} for secretion was established in a large variety of secretory systems (27). Thus, the omission of Ca^{2+} from the extracellular environment blocks the release of hormone in response to different secretagogues (25, 28–34). Reintroduction of Ca^{2+} into a Ca^{2+}-free medium produces a sharp increase in hormone release (24, 35, 36). This has been interpreted as arising from a rapid penetration of Ca^{2+} through the plasma membrane, since this divalent cation was added when the plasma membrane was in a state of increased permeability due to the omission of Ca^{2+} (23, 35). It appears, therefore, that stimulation promotes Ca^{2+} entry and this seems to be supported by the results obtained with Mg^{2+}. Increasing the extracellular concentration of Mg^{2+} inhibits hormone release in response to stimulation (28, 35, 37, 38). Replacement of extracellular Ca^{2+} by either Sr^{2+} or Ba^{2+} is effective in maintaining the stimulation-induced release of hormones (35, 38, 42). Furthermore, Ba^{2+} itself is a potent secretagogue, even in the absence of extracellular Ca^{2+} (35, 38–41). The possibility that Ba^{2+} acts by displacing intracellular Ca^{2+} should be considered, since Ba^{2+} interferes with ^{45}Ca binding by microsomes (43). Therefore, although Ca^{2+} is essential for the release process, different Ca^{2+} pools may be involved, depending upon the secretagogue used (34, 44).

Among the glands storing hormones in secretory granules, the parathyroid gland and the C cells of the thyroid gland seem to be a special case. The parathyroid hormone (PTH) and calcitonin control and maintain the normal levels of Ca^{2+} in plasma (45, 46). One of the most important sites of action responsible for this control is the bone, where PTH stimulates mobilization of Ca^{2+} and calcitonin inhibits this process. Furthermore, the plasma levels of PTH and calcitonin are indirectly and directly, respectively, related to the Ca^{2+} plasma concentration (45). Thus an increase in Ca^{2+} levels triggers calcitonin release whereas the release of PTH is depressed (45, 46). The opposite is true when the concentration of Ca^{2+} in plasma

is decreased (45, 46). Therefore, Ca^{2+} ions are the stimulus for calcitonin release. However, it is unknown whether there is an active calcium site in the plasma membrane of the C cell or whether Ca^{2+} can readily penetrate the cell and trigger release (45, 46). In the case of the parathyroid gland, an increase in the extracellular Ca^{2+} does not seem to be responsible for turning off the mechanism of release. On the contrary, a lag of 25–30 min is observed between the increase in Ca^{2+} levels and a 50% decrease in the circulating PTH levels (46). This probably suggests that Ca^{2+} acts on the regulation of the synthesis of the PTH (46, 47). There is, however, not enough available information to understand completely the cellular mechanisms which regulate PTH and calcitonin release.

Effect of Potassium Ions

As discussed above, increasing the concentration of K^+ in the extracellular environment depolarizes the endocrine cell (19–21). This results in an increase in membrane permeability to Na^+ and Ca^{2+} (16, 17). However, only Ca^{2+} is essential in the K^+-induced hormone release reaction. Thus, although the endocrine cell is depolarized by high K^+ in the absence of extracellular Ca^{2+}, hormone release is not triggered (25, 28, 30–35, 48). This effect of K^+ has been observed in many endocrine cells, including the neurosecretory terminals of the neurohypophysis (25, 35).

Sodium-Calcium Interaction, the Late Ca^{2+} Channel, and the Regulation of Intracellular Ca^{2+}

Experiments carried out on the adrenal medulla, the endocrine pancreas, and the neurohypophysis have shown that the omission of Na^+ from the extracellular space produces a sharp increase in hormone output (35, 44, 49). Moreover, after a short exposure to a Na^+-free solution, hormone release in response to stimulation is potentiated (25, 28, 35, 50). Therefore, it is likely that decreasing the extracellular concentration of Na^+ produces an increase in the $[Na^+]_i/[Na^+]_o$ ratio and, perhaps, a concomitant increase in the intracellular Ca^{2+} levels. This Ca^{2+} increase may be due to a decrease in the Na^+-dependent Ca^{2+} efflux mechanism. The existence of a Na^{2+}-dependent Ca^{2+} efflux has been shown in the squid axon and in the cardiac muscle (51). The potentiation of hormone release may also be due to an increased Ca^{2+} entry as a result of the removal of extracellular Na^+ (35, 50). The existence of a Na^+-Ca^{2+} antagonism has also been demonstrated in the squid axon and heart muscle (51). However, it is also possible that decreasing the $[Na^+]_o$ may increase $[Ca^{2+}]_i$ by inhibiting the cellular binding or sequestration of Ca^{2+} (44, 52). Therefore, it seems that conditions which produce an increase in the $[Na^+]_i/[Na^+]_o$ ratio will favor the accumulation of intracellular Ca^{2+} which may be necessary to trigger hormone release. The $[Na^+]_i/[Na^+]_o$ ratio can be increased either by removing extracellular Na^+ (28, 35, 44, 49), or by increasing intracellular Na^+ as a result of blocking the Na^+ pump by either K^+ removal or by ouabain treatment (35, 48, 53). Long exposure to Na^+-deprived solutions produced an opposite effect on hormone

release, namely, the inhibition of release upon stimulation (44, 48). This diminished response seems to be due to a progressive fall in the intracellular concentration of Na^+ during exposure to a Na^+-free medium (44). Furthermore, diphenylhydantoin, a drug which reduces the intracellular concentrations of Na^+, inhibits glucose-induced release of insulin (54).

Studies on the movement of Ca^{2+} in the squid axon have shown that the entry of this ion is biphasic: an early phase which seems to use the Na^+ channel and which is blocked by tetrodotoxin (51), and a later phase of Ca^{2+} entry which is tetrodotoxin insensitive and which is blocked by Mg^{2+}, Mn^{2+}, Co^{2+}, La^{3+}, and the Ca^{2+} antagonist methoxyverapamil (51). The Ca^{2+} entry through this channel is blocked by the same ions and substances which block neurotransmitter and hormone release (35, 51, 55, 56). Moreover, one of the characteristics of the late Ca^{2+} channel is its inactivation by prolonged depolarization (51). This type of inactivation seems to be responsible for the decrease in catecholamine output observed during K^+ stimulation (57).

Ca^{2+} is also probably involved in the termination of the release reaction. One or more of the following mechanisms may be responsible for this: (*a*) inactivation of the late Ca^{2+} channel; (*b*) extrusion of Ca^{2+} to the cell exterior; (*c*) cellular binding or sequestration of Ca^{2+}. Sequestration of Ca^{2+} by a Mg^{2+} ATP-dependent mechanism has been demonstrated in microsomal and mitochondrial fractions prepared from adrenal medulla (43). Therefore, these organelles may be responsible not only for the termination of the release reaction, but also for the low [Ca^{2+}] levels present during cellular resting conditions. Furthermore, these organelles may be the site of action of drugs like caffeine or theophylline, which are known to induce release in the absence of extracellular Ca^{2+} (58, 59).

Finally, it is worthwhile to stress that all this ion interplay reflects again a very close similarity between two apparently different processes, namely, contraction and secretion.

EXOCYTOSIS

In earlier studies it was suggested that the mechanisms of hormone release from glands containing secretory granules encompassed holocrine and apocrine secretion (60, 61) as well as other forms of release, for example: (*a*) extrusion of intact secretory granules into the cell exterior (62); (*b*) release from a "free hormone pool" (63, 64); (*c*) release by diffusion of hormones out of the granules into the cytoplasm (64, 65); or (*d*) release by dissolution of the granule membrane in the cytoplasm (66). The final common path proposed for the last three mechanisms of release was the diffusion across the plasma membrane of the hormones from the cytoplasmic sap into the cell exterior. However, in 1957 another mechanism of secretion was proposed by De Robertis & Vaz Ferreira, which suggested that secretion was by "reverse pinocytosis" (exocytosis), a mechanism whereby the membrane of the secretory granule fuses with the plasmalemma allowing the escape of the content of the granules to the cell exterior (67). Exocytosis seems to be the most logical

process of hormone release. If the disadvantages of the other forms of release are considered, it is immediately obvious that exocytosis is the simplest, the most economical, and the most efficient mechanism for releasing not only hormones but also enzymes and transmitter substances from the exocrine glands and nerve terminals respectively. If hormones were released from granules into the cytosol they would diffuse in all directions; large quantities of the hormones would be destroyed by enzymes present in the cytosol and only a fraction of the hormones would reach the cell exterior. Furthermore, the hormones in question would have to cross at least two membranes, those of the granules and that of the cell. Finally, if release were not by exocytosis, since the molecular size of the hormones which are stored in granules varies from very simple molecules like adrenaline to more complex ones like somatotropin or adrenocorticotropin, special transport mechanisms through these membranes, namely, the granule and the plasmalemma, would have to exist. There is now good morphological evidence for exocytosis in the adrenal medulla (67, 68); in the neurohypophysis (69); in the α (70), β (71), and δ (70) cells of the pancreas; and in the pituitary cells, namely, the thyrotroph (72), the corticotroph (73), the somatotroph (74), the mammotroph (75), the melanotroph (76), and even in the gonadotroph (77) where earlier studies failed to show the classical "omega" images of exocytosis (78). Exocytosis seems also to be the mechanism of release in exocrine glands (79), nerve terminals (27), and a variety of secretory tissues (27). Furthermore, biochemical studies carried out in some of these tissues have provided chemical evidence in favor of release by exocytosis. These studies have shown that hormones were released during stimulation along with other soluble granule components. In the adrenal medulla, for example, there is a concomitant release of dopamine-β-hydroxylase, chromogranin A, and ATP along with the catecholamines (44, 80–82). These soluble substances are quantitatively recovered in the effluent escaping from stimulated glands (44, 80–82). Furthermore, subcellular fractionation studies carried out on stimulated medullae have shown that the entire soluble content of the granule is discharged to the cell exterior (83–85), and that exocytosis is an all-or-none release process (86). Further work has demonstrated that, in the neurohypophysis, neurophysins I and II are released along with oxytocin and vasopressin (87, 88) and that stimulation of the pancreas brings about the concomitant release of insulin and the connecting peptide C (89). It has also been demonstrated that in the mast cell, a unicellular gland, the release of histamine is accompanied by a parallel release of heparin and granule protein (90). Biochemical evidence in favor of exocytosis obtained in other secretory tissues has also been published. These secretory tissues are the adrenergic neuron, the polymorphonuclear leucocyte, the platelets, and the basophile leucocytes (27). Other biochemical studies have demonstrated that the membrane components are retained within the cell after exocytosis (83, 85, 91, 92) and that, although large molecules, as for example dopamine β-hydroxylase (mol. wt. 290,000), leave the cell during exocytosis, no cytoplasmic marker enzymes like lactate dehydrogenase (44, 92–94), phenylethanolamine N-methyltransferase (95), and adenylate kinase (93) are detected in the effluents escaping from these stimulated tissues. This indicates that the vital feature of the cell, that is, the membraneous isolation of the cytosol from the extracellular environment, is maintained during exocytosis.

ORIGIN AND FATE OF THE SECRETORY GRANULES

Ultrastructural observations have suggested that secretory granules originate from the Golgi apparatus (75, 77, 96–103). The Golgi complex can be viewed as the membrane-bound compartment of the cell which is believed to be involved in the following functions (104): (*a*) the synthesis of certain polysaccharides; (*b*) the synthesis or attachment of carbohydrates side chains of glycoproteins (especially the addition of terminal sugars such as galactose, fucose, and sialic acid); (*c*) the assembling of secretory materials and formation of membrane-bound granules containing these materials; (*d*) the assembling of lysosomes. According to some investigators (105) the Golgi apparatus is part of the "endomembrane system" of the cell. This system is formed by the following components: rough endoplasmic reticulum $\longrightarrow$ smooth endoplasmic reticulum $\longrightarrow$ Golgi apparatus $\longrightarrow$ secretory granules. The arrows here indicate the direction of the "membrane flow," a term which has been introduced to describe the process of physical transfer of membranes from one cell compartment to another (105). This process of membrane transfer may or may not be accompanied by the concomitant transfer through this endomembrane system of secretory products, in this particular case, prohormones and hormones. Morphological evidence showing the packing of secretory products into granules in the stacked Golgi cisternae, especially in the maturing phase of the Golgi, has been published. This ultrastructural evidence has been collected from studies on all types of secretory cells of the adenohypophysis (75, 77, 96, 97); the α, β, and δ cells of the pancreas (97–100); the adrenal medulla (101, 102); the neurosecretory cells of the hypothalamus-neurohypophysial system (103); and in many other secretory systems in which secretory products are packed in vesicles (106).

Studies using pulse-labeling methods followed by autoradiography have also provided information on the synthesis, transport, and final packaging of secretory products. These experiments involved the incubation of the secretory tissues "in vitro" and the administration of a short pulse (5 min) of a radioactive amino acid, followed by autoradiography at different intervals of time postpulse. The results indicated that a high proportion of silver grains were always found on the surface of the endoplasmic reticulum at 5–10 min after labeling. The labeled material was then transferred to the Golgi apparatus, where the highest concentration was reached at 20–30 min postpulse. The radioactive material appeared largely concentrated in the secretory granules at 60 min postpulse. This pattern of movement of the label from the endoplasmic reticulum to the secretory granules has been observed in studies on somatotrophs (76), melanotrophs (107), β cells of the pancreas (99), and also in some exocrine cells (108, 109).

Although the results obtained with the use of autoradiography indicate the sequential movement of labeled material from one cell compartment to another, they do not indicate whether this transfer represents the movement of soluble or of membrane-bound protein or of both. Therefore, the results indicate only that some components of the granules are derived from the Golgi. Pulse-labeling procedures combined with biochemical techniques have demonstrated, at least in some cases, that the movement of labeled material represents the synthesis and transport of

hormones or their precursors. This seems to be true in the case of the pancreas where the movement of radioactive protein along this endomembrane system represents the synthesis and transport of proinsulin and its conversion to insulin (110). These experiments have suggested that the Golgi is probably the place where the conversion of proinsulin to insulin is initiated and that the transfer of material from the Golgi to the granules is an energy-dependent step (110). The synthesis of a precursor of oxytocin and vasopressin has been also suggested (103, 111). Furthermore, a suggestion has been made in favor of the origin of neurophysin I and oxytocin and of neurophysin II and vasopressin from common precursors respectively (112).

Pulse-labeling experiments followed by subcellular fractionation procedures carried out in perfused adrenal glands showed a similar pattern of endomembrane transport of [^{3}H]-leucine labeled protein, and newly synthesized chromogranins were detected in the perfusates as early as 30 min postpulse (113, 114). These pulse-labeling experiments have also shown the lack of labeled amino acids in the proteins of the membranes of secretory granules during the entire 4 hr of postpulse perfusion, this in spite of the fact that after 4 hr of perfusion the release of radioactive chromogranin could be readily detected (113, 114). These results indicate that the turnover of soluble granule components is more rapid than that of the membrane-bound components, which, in turn, suggests that granule membranes may be reutilized several times during the secretory cycle. This suggestion agrees with an earlier observation showing that the attachment of the granule membranes to the plasmalemma does not involve long-term incorporation (115) and that the granule membranes remain as discrete particles within the cells after catecholamine secretion (83, 116).

It seems, therefore, that the granule membrane proteins are not synthesized simultaneously with the granule content, although the morphological evidence discussed above does seem to indicate that the granules are derived from the Golgi complex. Consequently, it was important to obtain more biochemical information as to the origin of the secretory granules. To this end a method for the isolation of the Golgi complex from the adrenal medulla has been published recently (117). The chemical composition and the immunological properties of the Golgi membranes thus isolated have been compared to those of the chromaffin granule membranes (118). The marked biochemical differences observed between these membranes suggested that, if the secretory granules are derived from the Golgi as the morphological evidence seems to indicate, first, the granule membranes are unlikely to be derived by an unspecific "membrane flow," and second, the membranes must undergo considerable biochemical changes (membrane differentiation). The other possibility is, as suggested above, that after secretion the empty granule membranes return to the Golgi apparatus, fuse with it, and become recharged with newly synthesized hormones. Coated vesicles have been found in the proximity of the Golgi in electron microscopy pictures prepared from the adrenal medulla (119). Coated vesicles can also be seen in published electron micrographs prepared from other endocrine tissues (98, 120, 121). The interpretation given for the presence of coated vesicles in secretory tissues is that these vesicles have been derived from the granule membranes after exocytosis (122, 123). Therefore, a process of endocytosis and membrane retrieval would seem to follow exocytosis (122, 123).

The possible fate of these vesicles must also be considered. One possibility, already discussed above, is that they are reused in the formation of new secretory granules. Alternatively, it has also been suggested that these vesicles are incorporated into and digested by lysosomes. This process of digestion (crinophagy) of not only membranes but also intact secretory granules was first described in mammotrophs by Smith & Farquhar (75). Similar observations have been made in somatotrophs (76, 77), in gonadotrophs (76, 77), in thyrotrophs (76, 77), in α cells of the pancreas during spontaneous or experimental diabetes (124), in the neurohypophysis (125), in the normal adrenal medulla (126), and in pheochromocytomas (127). These autophagic bodies are observed mainly when a strong stimulus is removed or when hormonal secretion is suppressed. Therefore, it seems that this internal mechanism operates primarily when overproduction of secretory products occurs (76, 77, 124, 125, 127). Nevertheless, it is not known whether this is a controlled process or a blind one. It does not, however, seem to be the main factor regulating the fate of the membranes after exocytosis.

MECHANISMS OF RELEASE

Energy Requirement and the Possible Role of ATP

It has been shown that in many secretory tissues the release reaction is an energy-dependent process (128–131). Because secretion is blocked by inhibitors of oxidative phosphorylation and glycolysis (128–131) and because these two processes are important in the generation of ATP, it is possible that the energy requirement for the secretory process is in the form of ATP. However, it is not known whether the energy requirement is necessary to maintain a general cell function or whether it is needed in some of the steps involved in stimulus-secretion coupling.

Although the molecular events involved in the secretory process have not yet been completely elucidated, much information has been obtained by studying the effects of ATP on secretory granules isolated from the adrenal medulla. In the presence of Mg^{2+}, ATP produces structural changes in the chromaffin granules (132) and there is a simultaneous release of catecholamines, endogenous ATP, and soluble proteins (133). When ATP acts on chromaffin granules it is hydrolyzed by enzymes present in granule membranes, and part of the P_i so liberated is transphosphorylated to granule membranes (134). The effects of ATP on chromaffin granules can be blocked at either an early (ATP hydrolysis, transphosphorylation) or at a subsequent (conformational changes) step (135). On the basis of the above and other observations, Poisner & Trifaró proposed a hypothetical model for the molecular events involved in exocytosis (133). In this model the stimulation of the chromaffin cells induces an increase in intracellular Ca^{2+} (this being due either to increased Ca^{2+} entry or to liberation from intracellular sources). Then, Ca^{2+} may form a link between anionic groups (possibly phospholipids) of both granule and plasma membranes (133). It is known that Ca^{2+} can cause the aggregation of chromaffin and other secretory granules (136, 137). Moreover, it has been shown that Ca^{2+} causes the attachment of secretory granules of leucocytes to their membranes (138). ATP, which is released from the plasma membrane upon stimulation (139) and is perhaps

also freed from some other places within the cell, acts on chromaffin granules. During this interaction ATP is hydrolyzed by granule membrane enzymes; membrane protein and lipid are phosphorylated; and this is followed by the production of some conformational change (contractile event?) in the granule membrane leading to the release of soluble granule components. This hypothesis involves Ca^{2+} as one of the principal elements in membrane fusion. In connection with this it has been shown that ATP induces the synthesis of diphosphatidyl inositol in granule membranes (85, 140, 141). This lipid has great affinity for Ca^{2+} and, in some membranes, there is a direct correlation between Ca^{2+} binding and diphosphatidyl inositol content (142). It should be remembered that this hypothesis has been formulated on the basis of in vitro observations and although it accommodates all that is known about the release reaction, further work is necessary to see whether this mechanism will operate during release in vivo.

Nevertheless, ATP-induced release from secretory granules has also been demonstrated in other preparations such as zymogen granules (143), cholinergic synaptic vesicles (144), and posterior pituitary granules (145). The results obtained with ATP on granules isolated from the β cell of the pancreas are controversial (146, 147). This is perhaps because the experiments were carried out with "crude granule preparations," that is, fractions that were heavily contaminated with lysosomes, mitochondria, and microsomes (11). Furthermore, granule membrane phosphorylation by ATP has also been demonstrated in granules isolated from the adenohypophysis (148) and in zymogen granules (149). One of the criticisms of the hypothesis discussed above is the lack of effect of Ca^{2+} on chromaffin granules. However, it is possible that a factor which makes the granule ATPase sensitive to Ca^{2+} is lost or inactivated during the isolation of the granules. In this connection it has been shown recently that a protein fraction prepared from the cytosol of adrenal medullary cells sensitizes the granule to Ca^{2+} (150). In the presence of this cytosol factor, Ca^{2+} potentiates the ATP-induced release process (150).

Microtubules

It has been proposed recently that microtubules are involved in the mechanisms of hormone release in a variety of endocrine tissues (151–153). This hypothesis is based on indirect observations that agents which disrupt microtubules, such as colchicine and vinblastine, inhibit the release process. The first observation of this kind was made by Lacy et al (151), who reported that colchicine inhibits glucose-induced release of insulin from the pancreas. Lacy has suggested that secretory granules which are attached to microtubules, in contrast to those that are free in the cytosol of the β cell, are available for immediate release and that Ca^{2+} may trigger contraction or a change in the physical conformation in the microtubule system which results in granule extrusion (154). This hypothesis is subject to criticism for the following reasons. 1. The glucose-induced release of insulin from the pancreas is a biphasic event (155, 156): an immediate and acute release response (phase I) is followed by a late and long-lasting release response (phase II). Puromycin blocks by 50% the release during phase II without affecting phase I (157). Moreover, when pancreatic islets were prelabeled with [^{3}H]-leucine, the insulin released during phase

II was of higher specific activity than that collected during phase I (158). These observations suggest that insulin release during phase II is, at least in part, the result of either the conversion of proinsulin to insulin, or the release of newly synthesized insulin. These two phases of release are observed either when the pancreas is perfused or when pancreatic islets are perifused (155, 156). Colchicine blocks phase II of glucose-induced insulin release without affecting phase I (159). This differential effect of colchicine could not have been detected in the early experiments in which β-cell islets were incubated for 90 min (151). These results suggest that microtubules are not involved in the exocytosis process itself, and that the granules available for immediate release are not attached to microtubules. However, it still may be possible that, in this tissue, microtubules are involved in the intracellular transport of secretory granules from the Golgi (see above) to the periphery of the cell. 2. Lacy's suggestions that Ca^{2+} triggers contraction of microtubules also seems unlikely because Ca^{2+} blocks the polymerization of microtubules (160). In in vitro experiments, this divalent cation seems to be as effective as both colchicine and low temperatures in blocking microtubule polymerization (160). Furthermore, it has been postulated that the levels of free intracellular Ca^{2+} may control microtubule polymerization (161). If this is the case, microtubule polymerization should decrease during the release reaction, a condition in which an increase in free intracellular Ca^{2+} has been assumed (see above).

Therefore, a prerequisite for the study of these agents on the secretory process seems to be an adequate preparation of perfused glands in which exocytosis per se can be temporarily separated from the exocytotic process which follows intracellular transport. This prerequisite has not always been complied with, and many of the studies on hormone release from the adenohypophysis, neurohypophysis, and pancreas have been carried out in pieces or slices of tissues incubated in vitro and exposed to these agents for long periods of time (151, 153, 162–165). Therefore, in the presence of these agents a decrease in the amount of hormone collected during the stimulation was observed in some cases (151, 153, 162, 163); no changes (162, 164, 165) or even a potentiation of release was observed in others (164). Results obtained after long incubation periods in the presence of these agents are also difficult to interpret because of other effects of these drugs on living tissues. These effects are a decrease in production of ATP by colchicine and vincristine (166), the binding of colchicine to cell membranes (167), a drastic inhibition by colchicine and its analogues of nucleoside uptake (168), the tubocurarine-like effects of colchicine and vinblastine (169), and the anticholinergic effects of colchicine and vinblastine (170). This latter effect was observed in an endocrine gland, the adrenal medulla, in experiments in which perfused adrenal glands showed that the exocytotic release of catecholamines in response to either nicotine (152) or acetylcholine (152, 170, 171) was blocked by colchicine and vinblastine. However, catecholamine release induced by depolarizing concentrations of K^+ was unaffected (170, 171). This suggested an anticholinergic effect of these drugs, which was confirmed in experiments with perfused cervical ganglia (170). Furthermore, the lack of effect of colchicine on the histamine release from the mast cell induced by the Ca^{2+} ionophore A23187 suggests that in this tissue, as in the adrenal medulla, microtubules are not

involved in exocytosis (172). Therefore, the role of microtubules in the secretory process still remains to be elucidated.

Contractile Proteins

The similarities between the processes of secretion and contraction (23) taken together with the idea that excytosis is a true contractile event (133) raise questions about the presence and possible role of contractile proteins in secretory cells. In this connection the following observations have been made: (*a*) the presence of an actomyosin-like protein in adrenal medullary cells has been demonstrated (173, 174); (*b*) neurostenin, a protein which resembles actomyosin, has been isolated from the brain (175); (*c*) actin-like filaments have been detected by immunofluorescence in the β cell of the pancreas (176); (*d*) a protein of the same molecular weight and showing a similar finger printing pattern as muscle actin has been detected in the adrenal medulla (177); (*e*) a protein has also been isolated from this tissue which shows ATPase activity, electrophoretic mobility, molecular weight, amino acid composition, and electron microscopic appearance which closely resembles myosin (178); (*f*) in vitro interaction between muscle myosin and actin and chromaffin granules has been described recently (179); (*g*) a calcium-binding protein has been purified from the adrenal medulla (180); (*h*) actin has been shown in electron micrographs of glycerinated nerve terminals (181); and (*i*) cytochalasin B, a drug known to interact with microfilaments (182), has been shown to block release in some secretory tissues (163). Although the blocking effect of this drug might be due to its other metabolic inhibitor properties (183, 184), it is worthwhile to point out that the cytochalasin B blocks the polymerization of G actin into F actin (185). In consequence, it is possible that these contractile proteins are involved in one or more of the steps of secretion, for example, in the intracellular transport of secretory granules, in the extrusion of the granule contents, or in the formation of "coated vesicles" during membrane retrieval. The vesicle "coat" is perhaps formed by actin molecules. However, actin and myosin have also been isolated and characterized from a variety of nonmuscle cells (186) and these proteins are thought to be involved in the maintenance of cell shape, in cell motility, and in mitosis (186–188). Therefore, the possibility exists that the presence of actin and myosin in secretory cells is related to cell architecture rather than to the secretory process. Nevertheless, the possible role of myosin and actin in the secretory process should be fully investigated.

Membrane Fusion

Release by exocytosis involves the interaction of both the granule and plasma membranes. The discovery of a large amount of lysolecithin in chromaffin granule membranes (189, 190) has led to the suggestion that, during stimulation, this lipid is involved in the process of membrane fusion (190). Lysolecithin is not formed during stimulation, and it is present in the granule membranes during resting conditions (191, 192). Lysolecithin can cause the fusion of cell membranes (193), but its role in exocytosis has not yet been demonstrated. Furthermore, with the exception of chromaffin granules (189, 190) and the secretory granules of neurohy-

pophysis (194), there is no available information concerning the content of lysolecithin in other hormone storage granules.

As discussed above, Ca^{2+} may be another important factor in membrane fusion. In fact an electrostatic function for Ca^{2+} in exocytosis has been proposed recently (195, 196). The authors of this proposal have suggested that one possible function of Ca^{2+} ions in exocytosis is to reduce the potential at the surfaces of both the granule and cell membranes, thus decreasing the potential energy barrier for granule-membrane interaction (195, 196). This would enable contact or coalition to occur between the two membranes (195, 196). Whereas Ca^{2+} ions may play an important role in membrane fusion, the possible role of ATP and ATPase in this process cannot be ignored. In a review recently published on the mechanism of membrane fusion, a unitary theory of membrane fusion is proposed (197). This theory stresses the role of Ca^{2+}, ATP, and membrane-associated ATPase systems in the regulation of membrane changes responsible for fusion (197).

CONCLUSIONS

This review is an attempt to unify published observations about the secretory process in endocrine glands which store hormones in subcellular granules. One of the most interesting ultrastructural features of these tissues is the presence of secretory granules. Morphological evidence seems to indicate that secretory granules originate in the Golgi apparatus, and biochemical studies suggest that a selective process of membrane flow and differentiation must take place during granule formation. Granule storage not only allows these endocrine tissues to store large amounts of hormones in a relatively small volume, but also protects the hormones from intracellular degradation and provides a very efficient means of transporting and releasing fixed amounts (quantum) of hormones. In these tissues release is by exocytosis and this is an energy-dependent process which requires ionized Ca^{2+}. Therefore, an increase in intracellular Ca^{2+} seems to be a necessary requirement for triggering release. Increase in cytoplasmic Ca^{2+} may be produced by substances which interfere with the Ca^{2+} sequestration by intracellular organelles. However, it is more likely that an increase in Ca^{2+} entry under physiological conditions, through a specific Ca^{2+} channel, would account for this increase in intracellular Ca^{2+}. Where Ca^{2+} is needed still remains to be elucidated. Ca^{2+} may play a role in membrane fusion or it may be involved in some kind of contractile event leading to the release of hormones. Myosin and actin have been found in secretory tissues, but the role of these proteins in secretory cells still remains to be determined. Nevertheless, the close similarities which exist between two apparently different processes, namely, secretion and muscle contraction, suggest that the molecular events involved in secretion may be of similar nature to those involved in muscle contraction.

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