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The Secretory Cycle in the Adrenal Medulla

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In a broad sense, secretion encompasses all the events concerned with the synthesis, storage and release of products which are designed for some special function in the body. The entire process may be considered to compose a cycle consisting of three events—excitation, release of the product, and recovery. In the application of this concept to the adrenal medulla we consider excitation to consist of those events initiated by the release of acetylcholine at the splanchnic-adrenal medullary synapses which, in normal circumstances, results in the appearance of the secretory product, adrenaline, noradrenaline or both, in the extracellular space. The release process itself is considered to be those events involved in the transport of the products from the interior of the storage vesicle to the exterior of the cell. The combination of stimulation and release has been aptly termed "stimulus-secretion coupling" by Douglas (14, 19) thus implying that these events are separable although it is not possible at this time to state at which point stimulation ends and the release process begins. The term recovery is used in a limited sense here and refers only to those events required to restore the prestimulation levels of the released products and consists of both the resynthesis of the amines and the restoration of the storage vesicles.

Excitation of the Adrenal Medulla

Acetylcholine was shown to be the normal physiological mediator of excitation of the adrenal medulla (21) and both nicotinic and muscarinic receptors are present in the gland (22). Houssay and Molinelli (30) had previously shown that Ca^{2+} was necessary to induce secretion and the subsequent studies of Douglas (14) firmly established the importance of this ion in stimulus-secretion coupling.

The release response is coupled to excitation by a complex series of reactions that is poorly understood. In most studies the appearance of adrenaline or noradrenaline in the extracellular space has been used as an indication of effective excitation and it has not been possible to clearly differentiate the two events. However recent studies can clearly assign some early events to the excitation process itself. Acetylcholine, as well as other secretagogues, causes

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depolarization of the plasma membrane. Although the degree of depolarization is dependent upon the external concentration of calcium and sodium, nevertheless depolarization does occur in the absence of Ca^{+}, conditions under which no secretion occurs (15, 18). Banks (2) and Banks *et al.* (3, 4) have also found a requirement for external sodium for secretion in isolated bovine adrenal glands. Douglas (14) has reported that in no instance have they been able to evoke secretion without some changes in membrane potential or ion movement. Thus depolarization of the plasma membrane appears to be an early event in the excitation process but by itself is not sufficient to evoke secretion—Ca^{2+} is an essential requirement for coupling excitation to secretion.

Studies of enzyme secretion from leukocytes suggest that additional events are involved in the excitation process. Woodin and co-workers (64–66) found that stimulation of polymorphonuclear leukocytes with a specific secretory agent results in cytoplasmic solation and conversion of the normal orderly streaming of the secretory granules and other cell organelles into Brownian motion. The granules that come in contact with the plasma membrane adhere to the inner surface and their enzyme content simultaneously appears in the medium. In the absence of Ca^{2+} in the external medium the same cytoplasmic events occur with the exception that the granules do not adhere to the plasma membrane and no secretion occurs. Costero *et al.* (10) have recorded, by time lapse cinematography, alternating retraction and expansion of adrenal medullary cells grown in tissue culture and have also observed cyclosis and zeiosis of the cytoplasmic organelles, but no observations have been made in the presence of stimulating agents.

A metabolic source of energy that can be supplied either by glycolysis or by oxidative phosphorylation is required for excitation-release (38, 39, 57, 58). However it is not known whether the immediate requirement is for excitation, for release or for both.

The Release Process

Studies from several laboratories have established that release of catecholamines from the adrenal medulla occurs by exocytosis, a process in which the entire soluble content of the adrenal storage vesicles are released directly to the exterior of the cell leaving the membrane of the vesicle within the cell. This release presumably occurs by attachment of the vesicles to the plasma membrane resulting in changes in membrane permeability, or actual fusion of the two membranes with a direct opening to the exterior enabling the vesicle contents to leave the cell without passing through the cytoplasm. After release of their content, the storage vesicle membranes are detached from the plasma membrane and returned to the interior of the cell. Detailed evidence for this concept can be found in several reviews (14, 67) and only a brief account of the evidence will be presented here.

Practically all of the adrenaline and noradrenaline of the adrenal medullary cell is present in storage vesicles which early histologists have called chromaffin granules. In addition to the amines the storage vesicles also contain large amounts

of adenosine triphosphate (ATP), soluble proteins which collectively have been named chromogranins, the enzyme dopamine-β-hydroxylase which is present both as a soluble component of the vesicle content and a water-insoluble component of the vesicle membrane, Ca^{++}, Mg^{++}, and other small ions. On a dry weight basis the vesicles of the bovine adrenal medulla contain 21% catecholamines, 15% ATP and other adenine nucleotides, 35% protein, 80% of which is readily soluble in water, and 22% lipid which is almost exclusively present in the membrane (26). The vesicles of other species have similar compositions but may vary in the total percent of ATP in the adenine nucleotide pool (27). However the molar ratio of catecholamines to adenine nucleotides is approximately 4 in all species studied. There are also species differences in the amount of chromagranins present in the gland (63). The presence of ATP and chromogranin within the vesicle has led to the proposal that these components interact with the catecholamines to form a stable, non-diffusible complex which maintains the high concentration of amine within the vesicles.

Douglas and Poisner (16, 17) have shown that stimulation of the isolated adrenal gland results in the simultaneous appearance in the perfusion effluent of catecholamines and ATP or its breakdown products. The relative amounts of catecholamines and adenine nucleotides in the perfusion fluid was the same as that in the isolated vesicles suggesting that release of the content had occurred directly to the exterior of the cell. Subsequent studies by others have demonstrated that the chromogranins (5, 36, 37, 59), and dopamine-β-hydroxylase (68) are also concomitantly released with catecholamine upon stimulation of the gland and in the same relative amounts that are present in the water-soluble fraction of the gland. In these studies, proteins which are present in the cytoplasm (lactic dehydrogenase, phenylethanolamine-N-methyl transferase, tyrosine hydroxylase) or the mitochondria (succinic dehydrogenase, monoamine oxidase) were not released. In a series of studies *in vivo* Viveros *et al.* (69–71) found decreases in the soluble dopamine-β-hydroxylase content of the gland concomitant with the decrease in catecholamines resulting from insulin-induced secretion, but there was no change in the dopamine-β-hydroxylase content associated with the particulate fraction of the cell homogenates. This selective release of storage vesicle soluble protein and retention of storage vesicle particulate protein as well as cytoplasmic proteins can be satisfactorily explained only by release of the vesicle content directly to the exterior of the cell and retention of the vesicle membrane within the cell.

The studies of Viveros *et al.* (70) also provided additional information on the nature of the release process. The isolated catecholamine storage vesicles can incorporate exogenous amines (radiolabeled) from a buffered isotonic sucrose medium by a process which is stimulated by ATP + Mg^{++} and inhibited by reserpine and by sulfhydryl reactive reagents (which inhibit an ATPase activity of the storage vesicle membrane (9, 34)). During insulin-induced secretion from rabbit adrenal glands there is a commensurate decrease in the catecholamine content and the ability of the storage vesicle to incorporate exogenous amines. This observation suggested either that the parallel loss of catecholamine, ATP

and chromogranins resulted in a decreased ability of the vesicles to incorporate exogenous amines or that total release of the contents of a portion of the vesicle had occurred and that the "empty vesicles" were no longer able to incorporate exogenous amines. Evidence that secretion occurs by the latter process was provided by other experiments of Viveros *et al.* (72, 73). Catecholamine storage vesicles of high purity can be obtained by centrifuging a crude vesicle fraction through a continuous sucrose density gradient. The equilibrium density of the vesicles appears to be dependent upon their catecholamine content—those vesicles having a higher content being more dense. With dopamine-β-hydroxylase as a marker for the storage vesicle membrane Viveros *et al.* (73) found that the equilibrium density and the ratio of dopamine-β-hydroxylase to catecholamines in vesicles isolated from glands partially depleted of catecholamines after insulin administration was the same as that of vesicles obtained from unstimulated glands. If secretion occurs by the partial release of the contents of the storage vesicles one would expect a decrease in the equilibrium density and an increase in the ratio of dopamine-β-hydroxylase activity to catecholamine in the purified vesicles since all of the particulate enzyme activity is retained. However, if secretion occurs by total release of the soluble content one would expect the remaining vesicles to have the same buoyant properties and the same dopamine-β-hydroxylase to catecholamine ratio as vesicles from unstimulated glands; this indeed was observed, and, in addition the dopamine-β-hydroxylase associated with the membrane of the "empty vesicles" was found in a lighter portion of the density gradient as well as in the microsomal fraction of the homogenate. These studies have established that secretion occurs by exocytosis and that each of the vesicles respond to the stimulus by releasing its total content. In this sense the release of catecholamines by the storage vesicle is a quantal, "all or none" response.

Excitation-release Coupling

For the purpose of this discussion we wish to separate the mechanism of release from excitation and arbitrarily limit excitation to those events which occur in the cell, after interaction of the receptor with acetylcholine, which enable Ca^{2+} to reach its active site and all other events which result in a greater probability of the vesicles interacting with the sites of release. These events would consist of depolarization of the plasma membrane and the attendant movement of ions across the membrane, and increased mobility of the storage vesicles resulting from solation of the cytoplasm. The studies of Woodin and Wieneke (65) show that these changes occur in the absence of Ca^{2+} in the leukocyte. At this time it is not known whether relatively few specific releasing sites are present in the plasma membrane or whether the distribution is such that a high probability of release exists wherever the vesicles come in contact with the membrane during the excited state.

Viveros (67) has extensively reviewed the literature pertaining to "excitation-secretion" coupling and has proposed a general hypothesis for the event. Only a few of the salient features which led to this proposal are presented here.

Miledi and Slater (46) found that it was necessary for Ca^{2+} to be present in the external medium for secretion to occur from the giant synapse of the squid during impulse invasion. In a Ca^{2+}-free medium, if Ca^{2+} was injected into the axon immediately before the synapse, no secretion occurred, but if Ca^{2+} was applied externally, as little as 10^{-14} to 10^{-13} moles of Ca^{2+} per second was enough to maintain the secretory response to stimulation. Katz and Miledi (33) subsequently showed that Ca^{2+} must be present in the external medium during depolarization for secretion to occur. If the local application of Ca^{2+} was delayed to reach the membrane immediately after the spike potential no release of acetylcholine occurred.

Del Castillo and Katz (13) and Katz (32) have proposed a model for the release of acetylcholine at the cholinergic synapse in which the rate of collision between vesicles and plasma membrane is very high at all times but release occurs only in the improbable event of two reactive sites, one on the vesicle membrane and one on the plasma membrane, meeting and initiating a reaction which results in release of the transmitter. Excitation permits Ca^{2+} to move into the membrane and increase by a statistically large factor the number of effective collisions resulting in release of transmitter. Similar models have been proposed by Matthews (45) and by Woodin and Wieneke (66).

Rasmussen (56) has proposed a general hypothesis of cell secretion in which the stimulus promotes Ca^{2+} uptake and simultaneously increases the production of 3′,5′-adenosine monophosphate (AMP) which in turn may regulate intracellular Ca^{2+} inactivation and mobilization and may also activate a kinase system which is involved in the transport of the vesicles to the releasing sites. However, cyclic AMP and its dibutyryl derivative were completely ineffective in evoking secretion from the adrenal medulla when perfused through the gland at concentration as high as 10^{-3} M (35).

Whittaker (62) and Lacy *et al.* (42) have proposed a direct connection between storage vesicles and the external membrane through a microtubular system, the lumen of which would become enlarged in the presence of Ca^{2+}. One or several of the microtubule disrupting agents, colchicine, colcemide, vinblastine, vincristine, podophyllotoxin, and griseofulvin have been reported to block secretion of lysosomal enzymes (44, 55) and histamine (41) from leukocytes, insulin from the pancreas, I^{131} from previously labeled thyroid slices, and catecholamines from the adrenal medulla (53). However, only small numbers of microtubules have been observed in secretory tissues by electron microscopy and these in no special orientation. An alternate explanation for the action of these agents in inhibiting secretion may be that they impair the general internal mobility of the cell interfering with the movement of vesicle to the plasma membrane in some manner not directly related to their effect on microtubules.

Oka *et al.* (48, 49) and Poisner and Trifaró (54) have found that ATP-Mg^{2+} added to suspensions of adrenal storage vesicles cause release of the vesicle content to the medium. Poisner and Trifaró have proposed that stimulation releases Ca^{2+} and ATP bound to the plasma membrane and allows extracellular Ca^{2+} to enter the cell. Ca^{2+} would serve to link the vesicle membrane to the

plasma membrane. The vesicle membrane ATPase can simultaneously act on the released ATP, and this interaction results in some conformational change in the vesicle membrane which permits egress of the vesicle content through the areas of increased permeability in the vesicle and plasma membrane. In subsequent studies Lishajko (43) has shown that the ATP-Mg^{2+}-stimulated release does not occur in isotonic media containing less than 50 mM chloride ions, whereas secretion from perfused adrenal glands can occur in chloride-free media. Of 10 different organic and inorganic ions tested only Cl^-, Br^- and I^- will support ATP-Mg^{2+}-dependent release from the isolated vesicles (23). However, Ferris, Viveros, and Kirshner (24) have found an unidentified factor present in the adrenal medulla which will support release from isolated vesicle in sucrose media.

From these and other considerations Viveros has proposed that excitation-secretion coupling encompasses the following:

1. Interaction of the secretagogue with any of the many adrenomedullary receptors (nicotinic, muscarinic, and others), or direct depolarization, alters the conformation of the membrane such that there is an increase in conductance to sodium, calcium, and possibly other ions.

2. The increased membrane conductance to some ion other than calcium or other changes at the membrane results in cytoplasmic solation. As a consequence orderly movement of the cytoplasm and organelles stops and brownian movement of the particles through a medium of decreased viscosity starts. Random movement will direct some of the storage vesicles toward the plasmalemma.

3. The surface potentials of the plasma membrane and peripheral vesicles are reduced or abolished by the increase in calcium concentration in this area. As a result both membranes come into direct contact. This contact may be stabilized by a Ca^{2+} bridge linking the two negatively charged surfaces.

4. Additionally the Ca^{2+} may react with a specific site or sites on the plasma membrane resulting in an increase in the probability of fusion of the internal layer of the plasma membrane to the external layer of the vesicle membrane. Ba^{2+} and Sr^{2+}, but not Mg^{2+}, may substitute for Ca^{2+} in this reaction. The hypothetical site for activation of membrane fusion could be some specific molecular component of the membrane which, directly on binding to calcium, results in structural changes in the membrane, or Ca^{2+} could activate an enzyme system which may have such an effect (phospholipases, *cis-trans* isomerases, an actomyosin system which on contraction may expose the lipid phase of the membrane). The resulting area of fusion becomes a weak point in the vesicle membrane because of the local increase in tension resulting from the larger radius of curvature and the additive effects of vesicle and cell internal pressures.

5. The catecholamine storage complex starts to dissociate by an increase in the concentration of ATP, Mg^{2+} or the X factor (that is replaced by Cl^- *in vitro*) in the medium surrounding the vesicle. This activation of the storage complex, as a result of stimulation, may affect all vesicles in the cell or more probably is localized to its periphery. The activation of this step may result from a true increase in concentration of some of the required factors or because

of the vesicle moving into close proximity to the cell membrane where these factors may normally exist in high concentration. As a consequence, the pressure inside the vesicle increases and the vesicle membrane becomes more unstable. The increased intravesicular pressure will then result on the opening of the fused area into the extracellular space. The complete and rapid dissociation of the storage complex when exposed to the composition of the interstitial fluid may help flush out the vesicle content.

Recovery Phase of Adrenal Medullary Secretion

Release of catecholamines is accompanied by release of the entire soluble contents of the storage vesicles which include ATP, chromogranin and soluble dopamine-β-hydroxylase, and by disruption of the storage vesicle membrane. Restoration of the gland to the prestimulus state requires not only synthesis of the released amine but also synthesis of the released ATP and proteins as well as the repair of the old or synthesis of new vesicle membranes. There is little direct information on the rate-limiting steps in the recovery process or on whether the vesicle membranes are reused but studies by Viveros *et al.* (73) indicate that vesicle formation is not the rate-limiting step.

The temporal changes in the catecholamine content in the ability of the storage vesicle to incorporate exogenous amines and in enzymes involved in noradrenaline and adrenaline formation of rabbit adrenal glands following insulin induced secretion are shown in figures 1 and 2. Within 3 hr after the ad-

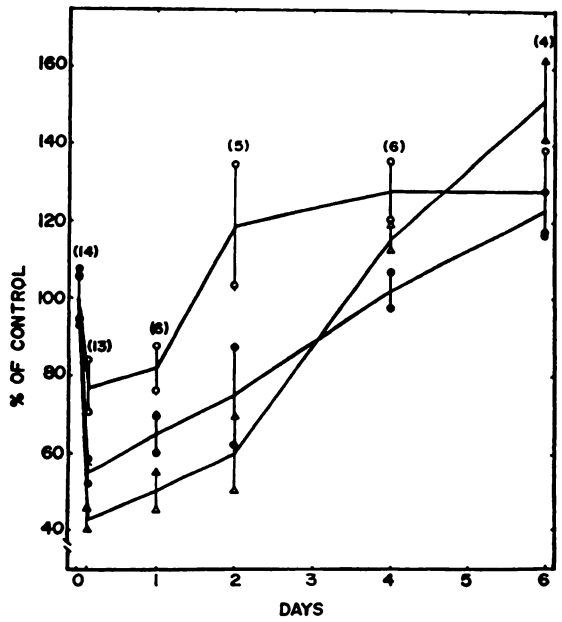

FIG. 1. Effect of insulin treatment on catecholamine content, dopamine-β-hydroxylase activity and uptake of ^{14}C-adrenaline by adrenal glands. Reprinted from Viveros *et al.*, Mol. Pharmacol. 5: 69–82, 1969. For details see reference 70.

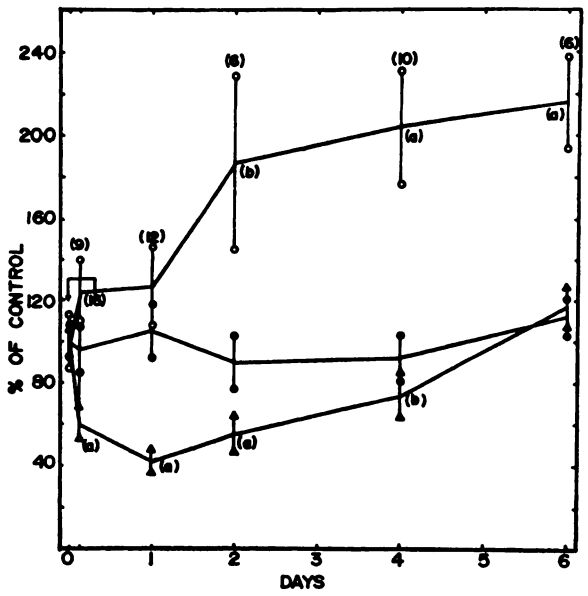

FIG. 2. Effect of insulin treatment on catecholamine content, tyrosine hydroxylase activity and phenylethanolamine-N-methyl transferase activity of adrenal glands. Reprinted from Viveros *et al.*, Mol. Pharmacol. 5: 69–82, 1969. For details see reference 70.

ministration of insulin there is a marked decrease in the amine content and the ability of the vesicles to incorporate exogenous amines. These parameters slowly recover to normal levels in 4 to 6 days. The change in dopamine-β-hydroxylase follow a different time course. At 3 hr there is a moderate decrease in activity which is accounted for by the loss of soluble enzyme from the storage vesicle. There is little or no change during the subsequent 20 to 24 hr but between 24 and 48 hr there is complete recovery of the enzyme to levels higher than normal. On the other hand, tyrosine hydroxylase shows little or no change for 24 hr but shows marked increases between 24 and 48 hr. Increases of tyrosine hydroxylase occur more rapidly in the rat after insulin treatment; within 24 hr there is a 50% increase in enzyme activity (60, 61). No changes in the levels of phenylethanolamine-N-methyltransferase are observed throughout the experimental period.

Analysis of the catecholamine and dopamine-β-hydroxylase content of the storage vesicles purified by isopycnic centrifugation through sucrose density gradients provided the information shown in table 1. Isopycnic centrifugation through sucrose density gradients enables one to separate intact vesicles from "empty" membranes and to distinguish between normal and partially depleted vesicles (72). The data in table 1 show the catecholamine and dopamine-β-hydroxylase content of purified vesicles (segments A + B of fig. 3) prepared at the indicated times after treatment of rabbits with insulin. The pattern of change is the same as that observed in the whole gland—the maximal decrease

TABLE 1

Time dependent changes in dopamine-β-hydroxylase and catecholamines in rat adrenal storage vesicles after insulin treatment

Time	DBO*	CA	DBO/CA
hr			
0	853 ± 92	58 ± 7	14.2
3	116 ± 17	10 ± 1	11.6
24	168 ± 14	10 ± 2	16.8
48	745 ± 33	18 ± 4	41.3
96–144	896 ± 147	49 ± 8	18.2

* Dopamine-β-hydroxylase (DBO) is expressed as nmoles octopamine formed from tyramine × 100 per gland pair per hour. Catecholamines (CA) are expressed as μg/gland pair. For experimental details see reference 73.

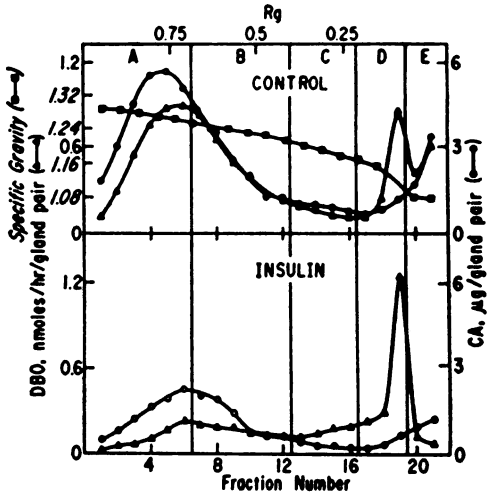

FIG. 3. Isopycnic centrifugation of the crude storage vesicle fraction obtained from control and insulin-treated animals. Reprinted from Viveros *et al.* Mol. Pharmacol. 7: 444–454, 1971. For details see reference 73.

was observed 3 hr after insulin administration (at which time the animals for the later time point are administered glucose to terminate the action of insulin) with little or no further changes at 24 hr. By 48 hr the vesicles have fully recovered their dopamine-β-hydroxylase content but have only partially recovered their catecholamine stores. The equilibrium density of the vesicles was the same as that of the controls but the shape of the curve was skewed to the right indicating a larger-than-normal proportion of partially filled vesicles. The ratio of dopamine-β-hydroxylase to catecholamine was more than twice that of the central or any of the other experimental time points. From 48 to 96 hr after treatment the vesicles recover their catecholamine stores and regain the normal enzyme to amine ratio. These data clearly indicate that either new vesicles are

synthesized or the old vesicles repaired including complete recovery of at least one soluble protein component long before they regain their amine stores.

The studies of Viveros *et al.* (73) indicate that at least some of the membranes of the vesicles which had released their contents are not reutilized but these studies did not determine whether vesicle formation required entire synthesis *de novo* of membranes. Studies of zymogen granule formation in the pancreas suggest that after secretion the old membranes or at least their protein components are reutilized to a considerable extent for the formation of new zymogen granules (31). However, studies of zymogen granule formation in the parotid gland (1) show that synthesis *de novo* of the protein components of the zymogen granule content and membranes occur concomitantly. After a pulse of radioactive amino acids the proteins of the membranes had the same specific radioactivity as the soluble proteins contained within the vesicles.

To what then is the long delay in the recovery of catecholamines due? Is this a reflection of slow catecholamine synthesis or is it a reflection of the ability of the vesicles to store catecholamines? In the studies described above, no measurements were made of the chromogranin content nor of the ATP. However it seems unlikely that the vesicles would regain one of the soluble protein components long before it regains the others. A number of studies have shown that the storage vesicles regain their ATP content concurrently with their catecholamines and it cannot be ruled out at present that recovery of the ATP stores is first necessary to enable formation of the storage complex.

The increase in tyrosine hydroxylase activity observed after stimulation of the adrenal medulla (60, 61, 70) and other sympathetic tissue (47), however, suggests that synthesis of the catecholamines *per se*, is the rate-limiting step. Estimation of the synthetic capability of the rabbit adrenal shows that the tissue has sufficient dopamine-β-hydroxylase to synthesize its total adrenaline and noradrenaline stores within 15 min and sufficient tyrosine hydroxylase to synthesize an equivalent amount of dopa within 20 hr when its activity is measured *in vitro* under near optimal conditions. The synthetic capability in the rat adrenal is higher and it contains sufficient tyrosine hydroxylase activity to replace the catecholamines within 5 hr (50). These estimations do not take into consideration regulatory controls *in vivo* discussed elsewhere in the symposium but they do suggest that the increase in tyrosine hydroxylase is of functional significance in the recovery of the catecholamine stores. Dairman and Udenfriend (12) have reported increased rates of synthesis of noradrenaline from tyrosine by adrenal glands of rats whose tyrosine hydroxylase levels had been increased by prior adrenal medullary stimulation.

Further evidence that the increased level of tyrosine hydroxylase contributes to the recovery of catecholamine stores after depletion comes from the studies of Patrick and Kirshner (51). Hökfelt (28) and Kroneberg and Schumann (40) found that denervation of the adrenal gland after insulin (28) or reserpine (40) treatment significantly delayed recovery of the catecholamine stores. Treatment with reserpine of rats which had their left adrenal gland denervated (51) (denervation prevents stimulation-induced increases in tyrosine hydroxylase)

resulted in an 84% catecholamine depletion of the intact gland and a 54% depletion of the denervated gland. Four days after reserpine treatment the intact gland had completely recovered their catecholamine stores while the denervated glands were still significantly depleted. Treatment of rat with acetylcholine, which increased the tyrosine hydroxylase activity in both intact and denervated glands (51), resulted in complete recovery of the catecholamine stores of both intact and denervated glands within 4 days after the last reserpine injection.

The levels of adrenal tyrosine hydroxylase dopamine-β-hydroxylase and catecholamines in rat are dependent upon both the age and the innervation of the adrenal gland (52). During growth from the 4th to the 12th week of age tyrosine hydroxylase activity increased 2- to 3-fold, dopamine-β-hydroxylase activity increased 2-fold and catecholamine levels increased 3- to 5-fold in intact adrenal glands. Denervation of the adrenal gland at 23 days of age markedly depressed the increase in enzyme activity and catecholamines to approximately one-half that seen in innervated glands. In a second series of experiments tyrosine hydroxylase, dopamine-β-hydroxylase and catecholamines increased 51, 33, and 57% respectively in intact adrenal glands. Denervating the left adrenal gland at 8 weeks of age completely prevented the increases seen in the innervated gland. These studies indicate that the adrenal gland has an inherent ability to develop its amine stores and synthetic capacity to certain levels in the absence of neural stimulation and superimposed upon this are adaptive increases to neurogenic stimulation.

What is the role of feedback regulation of catecholamine synthesis in the adrenal medulla? Several investigators who (7, 8, 29) used indirect methods of estimation have reported that during stimulation there is rapid resynthesis of secreted catecholamines; in some cases this amounted to as much as 50% of the initial amine content. However, other reports in which the amount of amine leaving the gland was directly measured showed that all of the amine which appeared in the venous effluent could be accounted for by losses from the gland (6, 20). No evidence for rapid resynthesis of secreted amines was found. With radioactive tracers several investigators (11, 25) have reported an immediate increase in the rate of synthesis of noradrenaline from tyrosine, but not from dopa, during adrenal medullary stimulation. However, the procedures do not enable one to determine the absolute amount of amine synthesized during the stimulation period. Since the specific activity of the amine of the stimulated adrenal was only 2 to 3 times that of the control, and since the turnover of amine in resting glands is slow with a half-life of 10 to 12 days (61) it appears that during the 1 hr experimental period only a very small fraction of the total amine stores would be replaced. The experimental evidence indicates that feedback regulation of amine synthesis occurs in the adrenal medulla and may be useful in promoting amine synthesis during period of mild stimulation. After more intense stimulation a second regulatory system controlling the amount of tyrosine hydroxylase is activated and results in increased levels of the enzyme. Thus two regulatory systems appear to be operative in the adrenal medulla; one responsive to feedback regulation for immediate control and one responsive

to increased neural stimulation which is designed to increase the total synthetic capability of the gland.

Conclusion

It is apparent that many questions remain unanswered in the secretory cycle of the adrenal medulla. Whether excitation causes solation of the cytoplasm and increased mobility of the storage vesicles and the role of microtubule, if any, in the secretory process has yet to be demonstrated. The nature of the excitation-release coupling and the function of Ca^{2+} remain to be clarified. The roles of feedback inhibitors and increases in tyrosine hydroxylase in regulatory catecholamine synthesis requires further documentations and the problems concerned with the synthesis of the storage vesicle membranes has barely been touched. These and many other intriguing questions should provide the stimuli to excite the release of much research energy during the next several years.

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