# REVIEW ARTICLE

# Mechanisms of Stimulus-Secretion Coupling in Adrenal Medulla

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... It is not to mere clinical experience that we must look for a revelation of the laws of disease. The laws of chemistry were not discovered in blazing fires or crumbling rocks; the laws of hydrostatics and hydraulics were not revealed in torrents, tides, or ocean currents, nor those of pneumatics and electricity in wind:, whirlwinds, and thunderstorms; much less could it be rationally expected that the laws of pathology should be discovered amid the much greater complexity and more multitudinous conflicts of elements presented to the physician at the bedside of a diseased or dying patient. It is in the laboratory, and by artificially contrived experiments that the clue has ever been spun and the torch lighted to guide through the labyrinths which hide the arcana of nature....

R. Cresson Stiles (1865)

Much of our present knowledge on the biochemical and morphological basis of the secretory process derives from studies on the adrenal medulla. The usefulness of this organ as a model for investigating both hormonal and neuronal release mechanisms was reviewed by Stjärne (1). The isolated perfused bovine adrenal gland has been more extensively used than adrenals of other species. Serrão and Da Costa (2) demonstrated that the cytological details as well as the catecholamine content of the isolated bovine adrenal medulla are well preserved even after prolonged perfusion (1–2 hr.) with physiological solutions. Since preservation of cellular structures in isolated organs is an indispensable condition for pharmacological investigations, the extensive use of the bovine adrenal gland as a model for studying the various aspects of neuroendocrine secretion seems justified.

Mammals are unique among vertebrates in that most chromaffin cells in the adult are confined within the adrenal medulla, which in turn is encapsulated by the adrenal cortex. The adrenal medulla derives from the neuroectoderm and is a neuroendocrine transducer, secreting its products in response to a neural input primarily from the preganglionic cholinergic fibers of the greater thoracic splanchnic nerves. Every chromaffin cell is said to be innervated (3). The adrenal

medulla has a rich dual blood supply, receiving blood that has drained the adrenal cortex and is rich in adrenal cortical steroids in addition to having its own direct arterial blood supply. Each chromaffin cell is said to be oriented with one end abutting on an arterial capillary and the other on a veinule (3), thus conveniently receiving biosynthetic precursors and releasing end-products, respectively. The anatomical relationship of the adrenal cortex and medulla is not without physiological significance. The role of the adrenocortical steroids in induction of medullary protein and phenethanolamine-N-methyltransferase synthesis and in determination of the relative distribution and anatomical disposition of epinephrine- and norepinephrine-containing cells of the medulla (4) was thoroughly reviewed by Pohorecky and Wurtman (5) and Wurtman et al. (6). However, although the cortex plays a significant regulatory role on the biosynthetic activity of the adrenal chromaffin cells, it appears to exert no immediate influence on the secretory process of catecholamines from the adrenal medulla (7, 8).

The details of the fine structure of the adrenal chromaffin cell of various species were described by several authors (9–22). The cytoplasm of the chromaffin cell contains a large number of what appear to be membrane-bound granules, 1000–3000 Å in diameter (23). The granules are reportedly bound by a unit membrane approximately 75 Å thick (4, 13), showing a trilaminar appearance (19). Fixation with glutaraldehyde and silver methenamine (10) results in the differential staining of three types of adrenal medullary cells: norepinephrine-containing cells showing black granules, epinephrine-containing cells showing light-gray granules, and a third cell type intermediate in appearance between epinephrine and norepinephrine cells. There is also evidence that epinephrine and norepinephrine granules may coexist within the same chromaffin cell, along with "precursor granules" whose content was chemically identified to be dopamine (9). The origin of the so-called chromaffin granules is a matter of conjecture. Small immature granules are said to be observed in the region of the Golgi apparatus (23), and "prosecretory" granules are reported to have been seen within the Golgi complex (17, 20); it has been suggested that the Golgi apparatus is directly involved in the formation of the chromaffin granules (9).

The adrenal medulla is the ancestral homolog of autonomic ganglia. The extensive investigations of Marley and Paton (24) and Marley and Prout (25) elucidated much of our present knowledge of the physiology and pharmacology of the splanchnic–adrenal medullary junction and the electrical properties of the chromaffin cell membrane. These authors studied the response of the adrenal chromaffin cells to nervous excitation and reported on properties such as threshold to nerve excitation, fatigue, spatial and temporal recruitment, and the effect of drugs (e.g., ganglionic blocking agents, eserine, and cocaine) on these parameters (24, 25). Douglas et al. (26, 27) further extended these observations by studying the effect of medullary secretagogues (e.g., acetylcholine, nicotine, pilocarpine, histamine, 5-hydroxytryptamine, angiotensin, and bradykinin) and antagonists (e.g., atropine and hexa-

methonium) on the membrane potential of adrenal chromaffin cells, as well as the influence of the ionic environment on this parameter. These studies demonstrated the presence of both nicotinic and muscarinic receptors on the adrenal chromaffin cell membrane (26).

## THEORIES OF CATECHOLAMINE RELEASE FROM ADRENAL MEDULLA[1]

The catecholamine hormones of the adrenal medulla are stored in high concentrations in the gland. In the rat, the adrenal medulla contains as much stored epinephrine as is secreted in 7 days (28, 29). The biochemical basis underlying the storage mechanism of the catecholamines was reviewed elsewhere (29–31). In brief, the catecholamines are believed to be bound in a nondiffusible form as a complex with adenosine triphosphate (ATP) and protein (32) or with ATP, calcium, magnesium, and RNA (33). The high concentration of catecholamines in the granules is believed to be maintained by an active uptake of amines across the membrane of the granules, which counteracts the loss of amines that occurs by outward diffusion (29).

In the past decade and particularly with the advent of the electron microscope and the introduction of biochemical techniques of subcellular fractionation, considerable progress has been made toward the understanding of the cellular storage of catecholamines and the manner of their release from the adrenal medullary cell (34–36). Several different mechanisms have been proposed to account for secretion from the adrenal medulla at a cellular level. As early as 1918, Cramer (37) proposed that the adrenal medullary granules were released as a whole from the adrenal gland (38). Round opaque particles, 300–700 Å in diameter and resembling chromaffin granules in appearance, were demonstrated in the canaliculi and intercellular spaces between the chromaffin cells of the adrenal medulla (19). In some cases where the particles were present at the free cell surface, small groove-like impressions occurred in the plasma membrane of the chromaffin cell (19). Similar findings of extracellular synaptic vesicles in the mouse heart were reported (39), as well as groove-like bays in the axolemma which were proposed to serve as points of exit of the granules from the nerve ending. Granule

extrusion from rat anterior pituitary cells following injection of leutinizing hormone-releasing factor was also recently demonstrated (40). Biochemical data do not support the theory of release of intact granules nor similar suggestions of apocrine or holocrine secretion from the adrenal medulla (41, 42), as will become evident from the ensuing discussion.

Based on cell fractionation studies, Hillarp (43) proposed the existence of at least three different pools of catecholamines in the adrenal chromaffin cell. One pool was the chromaffin granule which contained catecholamines in association with ATP with a molar ratio of 4:1. This ratio suggested that ATP served as the anion paired with the basic catecholamines, forming a complex which is an integral part of the intragranular mechanism of catecholamine storage (30). The second pool consisted of granules in which catecholamines were stored without ATP; the third pool consisted of the "free" cytoplasmic amines which, together with the pool of amines in the granules lacking ATP, comprised about 20% of all the catecholamines in the chromaffin cell, an amount that can sustain secretory activity for a prolonged time (44). Since ATPase activity was present in the chromaffin granule fraction (45), Hillarp (45) and Hillarp et al. (46) suggested that when the chromaffin cell is stimulated, the ATPase associated with the chromaffin granules may be activated in some way, enabling it to attack the ATP of the catecholamine storage complex, thus freeing the amines which would then diffuse or be transported out of the granules, into the cytoplasm, and ultimately out of the cell.

Blaschko and Welch (34) had earlier speculated that liberation of acetylcholine at the splanchnic–adrenal medullary junction might result in an increased permeability of the membrane of the chromaffin cell that would lead to a loss of "free" amines present in the cytoplasmic sap and that the role of the chromaffin granules was to replenish the cytoplasmic pool of catecholamines released during the secretory activity. In support of Hillarp's (45) suggestion of a critical role of granular ATP splitting in the release of catecholamines from their granules is the finding that ATP metabolites appeared in the adrenal effluent from stimulated glands (47, 48), and the observation that acetylcholine evoked catecholamine secretion by promoting calcium influx into the chromaffin cell (49)—a cation known to be an activator of certain ATPases and, consequently, to play a critical role in excitation–contraction coupling in muscle (50, 51). However, this hypothesis of intracellular release of catecholamines from the chromaffin granules followed by diffusion of the hormones to the cell exterior lost some of its credibility when Douglas et al. (52) noted that ATP metabolites present in the venous effluent from the adrenal were attributable to hydrolysis of ATP by endothelial enzymes and that, under appropriate experimental conditions, ATP is extruded unhydrolyzed to the cell exterior during catecholamine release from the gland (53). These observations led to the conclusion that splitting of intragranular ATP is not a critical step in the secretory process. Direct evidence against the hypothesis of intracellular release of catecholamines was provided by the finding that the secretion of the soluble contents of the chromaffin

granule, including the soluble intragranular protein chromogranin A (effective hydrodynamic radius approximately 62 Å), took place without simultaneous secretion of the cytoplasmic enzyme lactate dehydrogenase (hydrodynamic radius approximately 37 Å) (29, 54).

Although it is now generally recognized that the free cytoplasmic catecholamine pool demonstrated in cell fractionation studies results largely as an artifact of the homogenization procedure (29), and although the hypothesis of intracellular release of catecholamines has largely been superseded by other hypotheses, certain drugs such as acetaldehyde (55), dextroamphetamine (56, 57), l-amphetamine (58), and tyramine (58) are capable of releasing adrenal catecholamines from their storage sites into the cytoplasm of the chromaffin cell, from where the amines may diffuse to the extracellular space. Furthermore, spontaneous (resting) secretion of adrenal catecholamines is believed to utilize, at least in part, the intracellular release mechanism (58).

**Exocytosis Hypothesis**—This hypothesis was first proposed by De Robertis and Vaz Ferreira (18) in 1957 to explain their observations on structural changes in the adrenal medulla following stimulation of the splanchnic nerves. It was described later by De Robertis (59):

*When the gland is stimulated through the splanchnic nerve the first changes that occur in the catecholamine-containing vesicles (or granules) are the following: the granule attaches to the plasma membrane and it swells; at this moment there is no clear distinction between the clear zone on the outside and the dense granule inside; then there is a decrease in the amount of material contained in the granule. The material flows out but the membrane of the granule remains within the adrenal cell.*

This type of secretion was initially called "reverse pinocytosis" (18) but, more recently, the term "exocytosis" was introduced (60). Although this hypothesis received additional morphological support (12, 17, 19, 22, 61–65), it was disclaimed by others (66, 67). Electron microscopic evidence for exocytosis also has been reported for glandular tissue containing membrane-bound secretory granules other than the adrenal medulla (e.g., 64, 68–74).

Support for the exocytosis hypothesis derives heavily from biochemical evidence. That secretion from the adrenal medulla may take place by exocytosis is based mainly on the finding that several water-soluble constituents of the catecholamine storage granules appear simultaneously in the effluent from the gland. Thus, catecholamines, adenine nucleotides (48), the specific soluble protein chromogranin (75, 76), and soluble dopamine-β-hydroxylase (77) have been shown to be released from the adrenal medulla by various stimuli, sometimes in the same proportions in which they occur in the granules. Furthermore, neither water-insoluble constituents of the granules, such as cholesterol and phospholipids (78), nor cytoplasmic material, such as tyrosine hydroxylase, phenethanolamine-N-methyltransferase (77), or lactate dehydrogenase (29, 54), accompany the catecholamines secreted from the adrenal

medullary cells upon stimulation. The presence in the adrenal medullary chromaffin granule of a high content of lysolecithin (29, 79), a potent lytic agent (29, 80), led to the postulate that this lipid participates in the fusion of the secretory granule to the plasma membrane of the chromaffin cell during exocytosis (80).

The role of calcium ions in stimulus–secretion coupling will be discussed in a later section. However, one finding warrants mentioning since it provides a plausible basis for the explanation of the molecular mechanisms underlying exocytosis. Calcium appears to be almost universally involved as the link that couples excitation and secretion (81). Quarles and Folch-Pi (82) studied the effect of calcium on the distribution of gangliosides in an organic–aqueous biphasic system. They showed that when calcium is in the upper organic phase at concentrations below 5 m$M$ or above 160 m$M$, all of the ganglioside distributes in the organic phase. At calcium concentrations between 5 and 160 m$M$, the ganglioside partitions to the aqueous phase. When contaminated with protein, the ganglioside was induced by calcium to localize at the interface of the aqueous–organic phases. Since the chromaffin granules are composed of ganglioside and protein (29) and since the chromaffin cell consists of an aqueous (cytoplasm) phase and an organic (lipid and protein) phase (the plasma membrane), Simpson (83) proposed a similar situation to exist *in vivo*. Accordingly, a depolarizing stimulus to the chromaffin cell membrane would lead to an influx of calcium into the cell (49), resulting in a calcium concentration at the inner aspect of the cell membrane that is high enough to allow the collision between the granule (ganglioside) and the plasma membrane (organic phase) to persist, particularly since protein is present in both the granule and the cell membrane. Calcium could then activate membrane phospholipase A or granule lysolecithin, thus facilitating the fusion of the granule to the plasma membrane (79). Simpson (83) further proposed that when the stimulus to the chromaffin cell is terminated, the calcium concentration at the inner aspect of the plasma membrane of the chromaffin cell starts to decline; the granule (ganglioside) would repartition to the aqueous (cytoplasmic) phase and secretion would dwindle. To explain resting (spontaneous) secretion, Simpson (83) postulated that the very low calcium concentration found at the inner aspect of the chromaffin cell membrane at rest would increase the effectiveness of ganglioside (granule)–lipid (cell membrane) contact, in an analogous way to the *in vitro* situation described by Quarles and Folch-Pi (82).

The fate of the membranes of the chromaffin granules that remain in the chromaffin cell following discharge of their catecholamine content (64, 65, 84, 85) is a matter for conjecture. Although empty ghost granules have been seen electron microscopically in sections of medullary tissue from stimulated adrenal glands (59), it is not known whether these membranes become recharged with catecholamines, protein, ATP, and cations or whether they are phagocytized by the intracellular autophagic vacuoles and become broken down by lysosomal enzymes (29, 65). Evidence has been presented (64, 65, 71–74) for the adrenal chromaffin cells

as well as for the adenohypophyseal and neurohypophyseal cells that the granule membrane incorporated into the cell surface during exocytosis is removed by a process of vesiculation resembling micropinocytosis. In this process the exocytotic pits pinch off to form intracellular microvesicles, thus conserving not only the area of the plasmalemma but also its chemical characteristics associated with permeability, excitability, and the receptor function (64, 65). Kopin and Silberstein (86) suggested that the large norepinephrine vesicles in adrenergic neurons discharge their contents when the nerve ending is depolarized; once their soluble protein contents are exhausted, they may be transformed into the smaller storage vesicles and be concerned primarily with synthesis and storage of the transmitter reserve.

Although the exocytosis hypothesis for the release of neurotransmitters, hormones, and other macromolecules (80, 81, 83) has received considerable biochemical support, it does not explain a number of observations. Exocytosis certainly cannot be the mechanism by which the secretory products of the adrenal cortex, the fetal pancreas, or the parietal cells of the stomach are released, since the secretory products of these structures are not sequestered in membrane-bound granules (87–89). Furthermore, the exocytosis hypothesis cannot possibly account for the explosive and almost instantaneous release of transmitters, hormones, or macromolecules upon stimulation of cells containing these secretory products. Thus, catecholamines are released from the adrenal medulla within 1–2 sec. of splanchnic nerve stimulation (48), and epinephrine releases amylase from its storage granules in the parotid gland in less than 10 sec. (90). Likewise, insulin is released very rapidly from the pancreas following glucose stimulation (91). Schramm (80) emphasized that fusion of the zymogen granule with the cell membrane cannot explain the *rate* of enzyme secretion, and no experimental evidence indicates any temporal relationship between fusion, cleavage, and healing of the plasma membrane during exocytosis (64, 65, 71–74) and the rate of hormone, enzyme, or neurotransmitter secretion. Finally, the exocytosis hypothesis cannot explain the findings of Hubbard and Kwanbunbumpen (92) who demonstrated that there was no electron microscopic evidence of an increase in the number of discharging vesicles fused to the axolemma under conditions of increased quantal release of transmitter at the neuromuscular junction of the rat diaphragm.

Microtubule Hypothesis—The many serious and fundamental shortcomings of the exocytosis hypothesis prompted Whittaker (93–95) to propose the possible existence of a complex interconnecting system of tubules within secretory cells which may facilitate the discharge of secretory products to the cell exterior. According to Whittaker (95), "there might well be an intercommunicating system of fine tubules—hard to see by normal histological methods—composed of protein and another macromolecule that could open or close in response to ionic changes induced by action potentials." Such a system of microtubules would provide a possible pathway for release of all types of secretions, including the nongranule-bound secretions of the parietal cells, the adrenal cortex, and the fetal rabbit pancreas. In

addition, if the secretory tubules were to be considered as possessing a dynamic function as well as a structural one, they would provide a basis for the interpretation of the rate of egress of secretory products from secretory cells—perhaps the major shortcoming of the exocytosis hypothesis.

The possibility that secretion may take place within a structured environment of secretory tubules is supported by morphological observations. In negative staining, fine interconnections between isolated synaptic vesicles were demonstrated by Whittaker (93). Amsterdam and Schramm (96) showed that isolated zymogen granules are sometimes linked by thin tubules not visible in conventional sections of whole cells when examined under the electron microscope. Tubular connections may also account for the presynaptic projections and fine filaments sometimes seen in electron micrographs of nerve tissue (97). The existence of narrow tubules connecting the secretory granules to the plasma membrane was also proposed by Gray (97) and Sandborn (98). Electron microscopic evidence for the existence of neurotubules in axons was presented (98); De Iraldi and De Robertis (99) showed that these neurotubules appear to originate in the region of the Golgi complex in the soma and function in the rapid transport of material down the axon. Similar findings were reported by Davison (100). According to von Euler (101), "electron microscope pictures often show granules arranged in sequences, in rows with a kind of connecting tube or link between them" and that "practically every good picture shows at least a few granules which have such a connection between them." Von Euler (101) also observed "chains of two or three vesicles" in negatively stained preparations of suspensions of granules.

Kopin and Silberstein (86) described a situation in adrenergic neurons in which the secretory vesicles would move along, rather than within, microtubular structures which would serve as tracks to channel the vesicles toward the cell membrane of the terminal varicosities. In the adrenal cortex, there is evidence that the smooth endoplasmic reticulum forms an anastamosing system of tubular elements which plays a role in the extrusion of steroids to the cell exterior (88). Tubular connections running to the surface of the cell have been proposed to exist in cholinergic nerves (94), mast cells (102), and β-cells of the pancreas (91). The parietal cell of the stomach shows secretory canaliculi forming a sinuous tubular channel which opens into the lumen of the gastric gland (87). Furthermore, a system of cytoplasmic tubules, which is sometimes continuous with the canaliculus, has been identified in these cells (87).

Biochemical evidence for the existence of a microtubular secretory apparatus within secretory cells is rapidly accumulating, particularly since the introduction of colchicine, the vinca alkaloids, and cytochalasin-B as drug tools for the study of cell movement and intracellular transport systems. Colchicine binds specifically to microtubular protein (103), depolymerizes the microtubules of mitotic spindles, thus arresting mitosis, and unites with the protein subunits of neurotubules, causing a disruption of their organization (104). Similar effects were reported for the vinca alkaloids (105, 106). Vinblastine-induced precipitation of microtubular pro-

tein was reported by Marantz et al. (107). Cytochalasin-B is known to destroy neurofilaments specifically and to inhibit a wide variety of cellular movements, including changes in cell shape during differentiation and development (108). Cytochalasin-B was shown to interact with actomyosin from rabbit striated muscle, causing a decrease in viscosity of the actomyosin and an inhibition of actin-induced activation of heavy meromyosin ATPase activity by direct interaction with actin (109).

Reports are increasing on the inhibitory effects of colchicine, vinblastine, vincristine, and cytochalasin-B on secretory activity in secretory cells—a property invariably attributed to their disrupting effect on microtubules and microfilaments. Thus, colchicine and vinblastine inhibit the transport of amine storage granules in adrenergic neurons (110) and inhibit the release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves (111), a property also shared by cytochalasin-B (111). Release of catecholamines from the bovine adrenal medulla is inhibited by colchicine, vinblastine, and vincristine (112). Vincristine also was shown to inhibit the release of growth hormone and prolactin from the pituitary gland (113). Insulin secretion from the pancreas is blocked by colchicine (91), which also blocks thyroxine and iodine secretion from the thyroid gland (114, 115). Histamine release from mast cells (116) and leukocytes (117) and release of lysosomal hydrolases by phagocytes (118) are also inhibited by colchicine. Degranulation associated with phagocytosis (119) and melanin granule movement in melanophores (120) are likewise inhibited by colchicine. Cytochalasin-B inhibits latex-induced or zymosan-induced phagocytic activity in leukocytes, as well as phagocytosis of bacteria by polymorphonuclear leukocytes (121). Vincristine frequently produces signs of skeletal and smooth muscle toxicity consistent with diminished transmitter release (122).

In all of these systems, translocation of the secretory product or granule or vesicle movement appears to involve a microtubule system (123, 124) sensitive to the disrupting effect of colchicine, the vinca alkaloids, or cytochalasin-B—agents that labilize microtubular structures. On the other hand, the microtubule stabilizer deuterium oxide (125–127) has been shown to potentiate nicotine-induced catecholamine release from the adrenal gland (112) and histamine release from mast cells induced by compound 48/80 (116).

The proposal by Poisner and Bernstein (112) that the inhibitory effect of colchicine and vinblastine on evoked adrenal catecholamine secretion is due to disruption of secretory microtubules was contested by Trifaró et al. (128) and Douglas and Sorimachi (129, 130). They claimed that the inhibitory action of the alkaloids was attributable to an anticholinergic effect, since these agents inhibited the acetylcholine-induced release of catecholamines but did not block secretion evoked by high potassium concentrations (128–130). However, acetylcholine and potassium may act by different mechanisms to evoke catecholamine release from the adrenal medulla, as evidenced by the findings that lanthanum (131) and certain local anesthetics (132) block the secretory effect of acetylcholine more effectively than that of potassium. Furthermore, it is well documented that

colchicine can block the release of secretory products induced by noncholinergic stimuli in various tissues. For example, *in vitro* release of adrenocorticotropin from the adenohypophysis induced by high potassium concentration is blocked by colchicine (133). Similarly, glucose-induced release of insulin from isolated pancreatic islet β-cells is also blocked by colchicine (91). This alkaloid also blocks release of thyroxine and iodine from the thyroid induced by either thyroid-stimulating hormone or dibutyryl cyclic adenosine-3′,5′-monophosphate (cyclic AMP) by binding to a 6S colchicine-binding protein in the soluble fraction of homogenates (114), which is similar to the protein identified in other systems as a microtubular subunit (103, 134–138).

Recent investigations disclosed the presence of an actomyosin-like contractile protein in a number of secretory cells. Thus, a $Mg^{+2}$- or $Ca^{+2}$-activated ATPase was identified in isolated synaptic vesicles of rat brain (139, 140), and a similar ATPase was isolated from whole brain of the rat and cat (141) and characterized as having properties similar to those of muscle actomyosin and exhibiting an affinity for colchicine binding (142). A contractile protein was also isolated from human platelets (143) which was postulated to play a role in release of histamine and serotonin from these cells. Poisner (144) demonstrated the existence of an actomyosin-like protein in the bovine adrenal medulla, which could participate in the secretory mechanism of catecholamines.

Such findings have generated a number of theories relating the presence of actomyosin-like activity, the existence of microtubules, and the secretory process (78, 91, 104, 112, 114, 145). Poisner and Bernstein (112) crystallized these theories into a unitary thesis which postulates that "microtubules assist in the transport of secretory granules to the cell surface by a mechanism involving mechanicochemical transduction comparable to the contraction of actomyosin, and the final discharge mechanism also includes a contractile process with an actomyosin-like protein in the granule membrane, perhaps with the microtubules serving to orient the membranes at the surface to permit the active ejection of secretory product."

In view of the almost universal requirement for calcium in release of secretory products (81 and to be discussed later), Whittaker (94), referring to the possible role of microtubules in secretion, postulated that "if the walls of such tubules were made up of a macromolecule which changed configuration in the presence of calcium ions in such a way as to enlarge the lumen of the tubules, a basis might be provided for the well known essential role of calcium ions in excitation-secretion coupling." Because of the similarities between microtubule protein and actin (104), it is entirely possible that the "macromolecules" postulated by Whittaker (94) may actually constitute the contractile actomyosin isolated from secretory cells (139–144).

Rahwan (146) and Rahwan *et al.*[2] recently demonstrated by electron microscopy the existence of tubular connections between catecholamine granules in sec-

tions of mouse adrenal medulla. These authors advanced a theory for secretion from the adrenal medulla based on the postulate that the hormones may exist in the form of intratubular *droplets* within the chromaffin cell. A stimulus to the adrenal chromaffin cell would lead to an influx of calcium from the extracellular space (81) or translocation of calcium from intracellular pools (57) into the cytoplasm. The free intracellular calcium, either as ionic calcium or as a complex with intracellular phosphate (147), would activate an actomyosin-like protein in the contractile membranes of the secretory tubules, possibly by binding to a troponin-like factor (145), resulting in a peristaltic wave which would end in the egress of the catecholamines to the exterior of the cell (146)[2]. The isolation of intact chromaffin *granules* from homogenates of adrenal medulla does not necessarily militate against the postulate of the existence of the catecholamines within the chromaffin cell in the form of intratubular *droplets* (146)[2], since it is conceivable that when the gland is homogenized the fragmented tubules may undergo healing and vesicle formation in a manner analogous to the formation of synaptosomes[2].

## ROLE OF CALCIUM IN STIMULUS–SECRETION COUPLING IN ADRENAL MEDULLA

A number of comprehensive reviews covered various aspects of the role of extracellular calcium in the release of neurotransmitter substances, hormones, and other secretory products (44, 47, 81, 83, 145, 148, 149). The role of intracellular calcium in stimulus–secretion coupling has only recently been recognized and will be reviewed here. In general, calcium appears to play a critical and almost universal role in the release of secretory material. Furthermore, a relationship between calcium and cyclic AMP as intracellular messengers in secretion was discussed by Rasmussen (124). In the adrenal medulla, however, stimulation of the chromaffin cells does not result in an increase in intracellular cyclic AMP concentrations (124), and the role of this cyclic nucleotide, if any, in catecholamine secretion from the adrenal medulla is not known (124) despite the dependence of the secretory mechanism of this gland on calcium (81).

**Role of Extracellular Calcium**—Calcium-deprived perfusion solutions depress or abolish the secretory response of the adrenal medulla to a variety of secretagogues including acetylcholine, carbachol, other nicotinic and muscarinic agents, histamine, serotonin, polypeptides such as bradykinin and angiotensin I, some sympathomimetic amines, ouabain, and excess potassium (33, 150–159). Furthermore, Douglas and Rubin (150) showed that the secretory response of the adrenal medulla to acetylcholine varies directly with the concentration of calcium in the perfusion medium. Acetylcholine, potassium, histamine, angiotensin I, bradykinin, serotonin, nicotine, carbachol, and pilocarpine have been shown to depolarize the chromaffin cell membrane (26). An increased influx of radiolabeled calcium into chromaffin cells occurs during stimulation (49, 160, 161), and Douglas and Rubin (150, 162–164) proposed that acetylcholine owes its stimulant effect on

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the chromaffin cell to some action increasing the permeability of the plasma membrane to calcium in the extracellular fluid. The effect of acetylcholine on the permeability of the chromaffin cell is thought to occur through depolarization (44, 148). However, the finding that acetylcholine can still evoke catecholamine secretion from the adrenal medulla perfused with a sodium-free medium or a medium containing isotonic potassium sulfate indicates that depolarization is not tightly coupled with secretion as long as calcium is present in the extracellular environment (44, 148, 162). Additional evidence that depolarization and secretion can be dissociated was provided by Douglas *et al.* (27) who demonstrated that acetylcholine or excess potassium still depolarized the chromaffin cell when the extracellular fluid was calcium free or contained excess magnesium—conditions that inhibit secretion (150, 157, 162). However, in the work of Douglas *et al.* (27), the depolarization of the chromaffin cell in response to acetylcholine in the absence of extracellular sodium was not totally abolished but fell to about 30% of the control value. Moreover, prolonged perfusion with sodium-free medium abolishes the secretory response of the adrenal medulla to carbamylcholine and to high potassium concentrations (165)—agents that depolarize the chromaffin cell membrane (26). Although Banks *et al.* (165) suggested that sodium has some regulatory effect on the entry of calcium into chromaffin cells during stimulation, Rubin (81) argued that the prolonged perfusion with sodium-free medium used by Banks *et al.* (165) may result in a nonspecific deleterious effect to the chromaffin cell rather than a specific effect on calcium entry. However, sodium-deficient perfusion solutions cause an increase in spontaneous adrenal medullary secretion (150, 162, 165), and sodium deficiency has been shown to increase the entry of calcium into cells (165, 166).

An increased uptake (49) and exchange (160) of calcium by the chromaffin cell occurs during acetylcholine stimulation, and magnesium markedly inhibits calcium exchangeability between the gland and the perfusion medium (161) and also inhibits medullary secretion (162). Likewise, local anesthetics block the influx of calcium as well as the secretory response of the adrenal medulla (57, 161, 167). Rubin *et al.* (161) suggested that local anesthetics and magnesium interfere with calcium entry into the chromaffin cell by competition at a common membrane site. However, Rahwan *et al.* (57) presented evidence that magnesium may also interfere with the action of calcium at an intracellular calcium receptor site in the adrenal chromaffin cell.

**Role of Intracellular Calcium**—Despite substantial evidence of the importance of extracellular calcium in stimulus–secretion coupling in the adrenal medulla, in some instances drugs have been shown to evoke release of catecholamines from the perfused adrenal gland in the absence of calcium in the perfusion fluid. Thus, reserpine (33, 152), tyramine (152), phenylethylamine (152), barium (163), potassium thiocyanate (168), strontium (163), caffeine (56, 57, 169), aminophylline (169), acetaldehyde (55), chlorpromazine (56, 57), and dextroamphetamine (56–58) have all been shown to release catecholamines from the adrenal gland in the

absence of extracellular calcium. Likewise, the reintroduction of calcium to the perfusion medium following perfusion with calcium-free medium evokes catecholamine release from the adrenal medulla (150, 162), despite the fact that under normal conditions excess calcium added to a perfusion medium already containing calcium does not augment secretion in the absence of a depolarizing agent (163).

The dissociation of extracellular calcium and the secretory effect of some secretagogues do not militate against the role of calcium in secretion. Indeed, it is becoming increasingly apparent that intracellular calcium may be of importance in stimulus–secretion coupling. In animal cells the concentration of calcium in the cytoplasm is in the range of $10^{-5}$–$10^{-8}$ $M$ (124, 170–172). The demonstration by Borowitz *et al.* (173) that the adrenal chromaffin cell has a high content of calcium distributed among the various intracellular organelles raised the possibility that this intracellular calcium may play a role in catecholamine secretion in much the same way as has been demonstrated for muscle contraction (50). Thus, agents that release catecholamines from the adrenal medulla in the absence of extracellular calcium may still be dependent on a critical intracellular calcium pool for their secretory effect. In fact, Rahwan and Borowitz (56) and Rahwan *et al.* (57) showed that, under calcium-free perfusion conditions, chlorpromazine induced catecholamine release from bovine adrenal glands by mobilizing mitochondrial calcium reserves, caffeine evoked catecholamine secretion by mobilizing calcium from the endoplasmic reticulum as well as the mitochondria, and dextroamphetamine released adrenal catecholamines initially by a calcium-free mechanism followed by an enhanced secretory effect consequent upon mobilization of calcium from the endoplasmic reticulum. Furthermore, catecholamine release induced by chlorpromazine, caffeine, and dextroamphetamine in calcium-free medium generally paralleled $^{40}Ca$ and $^{45}Ca$ efflux from radiocalcium-labeled adrenal glands (57), suggesting that the calcium released to the exterior of chromaffin cells under these conditions represents in part the free intracellular levels of calcium that mediate catecholamine secretion (57) and in part the intragranular calcium involved in the catecholamine storage complex (174). The finding that magnesium significantly inhibited catecholamine secretion from the adrenal medulla induced by chlorpromazine, caffeine, or dextroamphetamine under calcium-free perfusion conditions (57) offers rather conclusive evidence that mobilization of intracellular calcium by these secretagogues is the triggering event in stimulus–secretion coupling and establishes an intracellular site for competition between calcium and magnesium (57).

Jaanus and Rubin (175) demonstrated that the total calcium content of the adrenal cortex may be divided into three roughly equal fractions: extracellular, cellular extractable, and cellular nonextractable. The extracellular calcium pool was presumed to be of importance for maintaining the more critical cellular calcium pools. During prolonged perfusion with calcium-free solution, approximately one-third of the total calcium content of the cortex was still retained by the gland. The locus of this nonextractable cellular calcium was assumed to

be the mitochondria and could be depleted only by treatment of the gland with 2,4-dinitrophenol, an uncoupling agent that releases calcium from the mitochondria (176). The cellular extractable calcium pool was assumed to be bound to or near the surface of the cortical cell or localized in some readily exchangeable intracellular site (175). The role of these different calcium pools in adrenocorticotropin-induced corticosteroid secretion was discussed by Jaanus and Rubin (175). The secretory response of the adrenal cortex to low concentrations of adrenocorticotropic hormone was found to be dependent upon extracellular calcium (177, 178), whereas high concentrations of adrenocorticotropin evoked corticosteroid release in the absence of extracellular calcium (179). It has been reported that the secretory effect of adrenocorticotropin on the adrenal cortex is dependent upon some critical intracellular calcium pool (175, 179).

Additional evidence for a role of intracellular calcium as a coupling link in the secretory process was provided by Williams (180). He showed that secretion from the thyroid gland induced by thyroid-stimulating hormone may be dependent upon mobilization of intracellular calcium.

To emphasize further the emerging significance of intracellular calcium in cellular processes, it has been reported that in certain tissues where adenyl cyclase and cyclic AMP mediate the effect of a specific peptide hormone, although the hormone-induced stimulation leads to an influx of calcium into the cells, the addition of extracellular cyclic AMP does not stimulate calcium uptake even though it mimics hormone action (124). Such findings indicate that the action of the cyclic nucleotide is either independent of calcium or utilizes an intracellular calcium pool (124). Evidence that cyclic AMP causes mobilization of calcium from intracellular pools has been presented by numerous investigators (181–184). Based on this and other information, Rasmussen (124) postulated that the increase in intracellular cyclic AMP, subsequent to interaction of a hormone with adenyl cyclase, would result in mobilization of calcium from one or more intracellular pools and/or prevention of uptake of cytoplasmic calcium into intracellular organelles.

Although the adrenal medullary chromaffin cell has been impaled by microelectrodes (26, 27), there is as yet no report on the effect of intracellularly injected calcium on catecholamine secretion. Such studies should provide direct evidence for the role of intracellular calcium in stimulus–secretion coupling. Miledi and Slater (185) found calcium to be ineffective in evoking release of transmitter when injected intracellularly into the squid giant axon. In their study, Miledi and Slater (185) deprived the squid giant synapse preparation of calcium and then tested the effect of ionophoretic application of calcium at different spots along the synapse. The transmitter-releasing effect of calcium was found to be very sharply localized at specific spots along the stretch of presynaptic axon lying adjacent to the postsynaptic axon. To test the effect of intracellularly injected calcium, the pipet was inserted into the presynaptic axon at a *single* spot which was previously shown to be sensitive to extracellular application of

calcium. When injection of calcium into the presynaptic axon resulted in no release of transmitter, Miledi and Slater (185) concluded that for calcium to evoke transmitter release the cation acts through a membrane reaction whose reactive sites are accessible only from the outside of the membrane. However, Miledi and Slater (185) conceded that such a conclusion "remains tentative because time was too short to test its validity fully." These authors (185) further stated that: "Usually we could not test more than one 'intracellular spot,' and it is clear that many spots must be tested in view of the sharp localization required for effective ionophoretic application of calcium from the outside." The question of whether intracellularly injected calcium can release secretory products remains to be answered.

The importance of calcium ions in spontaneous (resting) secretion of catecholamines from the adrenal medulla is not clear. Although some investigators reported spontaneous catecholamine secretion to be very low in the absence of calcium in the perfusion fluid (150, 154, 158), others showed that resting secretion from the adrenal medulla was unaffected when the perfusion fluid was switched from one containing 2.2 mM calcium to one containing no calcium (57). The spontaneous secretion of catecholamines from the adrenal medulla is presumed to reflect what is released by exocytosis initiated by spontaneous release of acetylcholine at the splanchnic–adrenomedullary junction as well as what diffuses out of the chromaffin granules and into the cytoplasm (58). This was shown to be the case by Schneider (58), since addition of hexamethonium to the fluid perfusing adrenal glands in a concentration that completely blocks the effect of acetylcholine released by the preganglionic cholinergic fibers resulted in approximately a 40% reduction of spontaneous catecholamine release, equivalent to the reduction observed when calcium was eliminated from the perfusion fluid. These results indicate that extracellular calcium has no effect on spontaneous catecholamine secretion other than that mediated by inhibition of exocytosis (58).

Spontaneous secretion clearly does not cease in the absence of extracellular calcium and, furthermore, is not abolished by either local anesthetics (57, 161) or magnesium (57, 162) whether or not calcium is present in the extracellular fluid. The finding that magnesium does not inhibit resting secretion when adrenal glands are perfused with calcium-free medium (57) casts doubt as to the importance of intracellular calcium in spontaneous catecholamine secretion, particularly since magnesium was shown to compete with calcium at an intracellular site (57). Furthermore, ouabain, which raises intracellular calcium levels by presumably inhibiting calcium efflux from cells, does not potentiate resting secretion from the adrenal medulla in the absence of extracellular calcium (154). On the other hand, Douglas and Ishida (186) reported that cooling increases resting secretion of vasopressin from the neurohypophysis perfused with a calcium-free medium, and cooling has been shown to inhibit calcium uptake by intracellular organelles including the endoplasmic reticulum of the adrenal chromaffin cell (168). Since calcium uptake by intracellular organelles is presumed to be one mechanism for termination of secretory

activity (discussed later), it is not unlikely that cytoplasmic calcium may play a role in spontaneous secretion and that interference with intracellular sequestration of this cation may enhance resting secretion. Furthermore, calcium was shown to be released from the adrenal chromaffin cells (57) and from isolated chromaffin granules (187) during spontaneous secretion even in the absence of extracellular calcium (57). Whether this extruded calcium is involved in resting secretion, as has been postulated for evoked adrenomedullary secretion (57), must await further research.

## PARALLELISM BETWEEN STIMULUS–SECRETION COUPLING IN ADRENAL MEDULLA AND EXCITATION–CONTRACTION COUPLING IN MUSCLE

Over the past few years, the striking similarities between the processes of stimulus-secretion coupling in secretory cells and excitation–contraction coupling in muscle have become increasingly apparent (57, 81, 145). Thus, all secretory systems studied have shown that the release of the secretory product is critically dependent on extracellular (81) or intracellular (57, 175, 180) calcium ions. Furthermore, studies of many secretory systems, including neurons and endocrine and exocrine cells, have shown that discharge of stored product does not occur when synthesis of ATP is blocked (188-190) or when granule ATPase is inhibited (78, 191). These facts, in addition to the isolation and characterization of an actomyosin-like contractile protein from a number of secretory cells (139-144), have emphasized the similarities between secretion and muscle contraction which were recently reviewed by Poisner (145).

The early finding that the adrenal chromaffin granules contained ATP and ATPase activity (45), coupled with the demonstration that the ATP-Mg$^{+2}$-dependent pump which concentrates catecholamines in these granules (30) has many characteristics of the calcium pump in muscle (192), led Hillarp (45) to suggest that when the chromaffin cell is stimulated, the ATPase associated with the chromaffin granules may hydrolyze the ATP of the ATP-Mg$^{+2}$ intragranular storage complex, thus releasing the catecholamines from the granules. Although this hypothesis was refuted on other grounds (discussed previously), its significance also was made doubtful when it was demonstrated that calcium did not evoke catecholamine release from isolated adrenal medullary chromaffin granules (78). However, Poisner (145) speculated on the failure of calcium to facilitate release of catecholamines from isolated chromaffin granules in vitro as being possibly due to the absence of an active calcium-sensitive protein comparable to the troponin–tropomyosin complex in muscle (193). The possibilities that such a protein may constitute an integral part of the membranes of the tubules involved in secretion and that it may be lost during the homogenation and isolation procedures (145) are thought provoking. Furthermore, the finding that radioactive calcium entering the chromaffin cell during stimulation readily penetrates the chromaffin granule (160) is suggestive of a pooling of calcium in the vicinity of the secretory elements of the chromaffin cell for the purpose of triggering catecholamine release.

## MECHANISMS OF TERMINATION OF SECRETORY RESPONSE OF ADRENAL MEDULLA

Since parallels between secretion and muscle contraction have been described with respect to release of secretory products from whole cells and from isolated granules, and since the molecular mechanisms of release of catecholamines have, in fact, been proposed to be contractile in nature[2] (84, 112, 145), further biochemical similarities between secretion and contraction can be outlined.

One very important aspect of the contractile system in muscle is that the concentration of ionized calcium in the myoplasm is maintained at low levels, and the sarcoplasmic reticulum has been shown to possess an ATP-activated uptake mechanism which reduces free intracellular calcium levels (194). It would be expected that secretory cells would also have some active mechanisms for keeping cytoplasmic levels of free ionized calcium low. Indeed, Borowitz (195) demonstrated that calcium is readily bound to subcellular organelles of the adrenal medulla. Poisner and Hava (168) reported that microsomes from the adrenal medulla can bind calcium in the presence of ATP and that this ATP-activated binding of calcium is inhibited by agents [e.g., quinidine, amytal, thiocyanate, strontium, barium, and p-hydroxymercuribenzoate (168)] that are known to block calcium uptake by muscle microsomes (196, 197).

Recently, Haugaard et al. (198) demonstrated an active uptake of calcium by mitochondria in cardiac muscle, and they speculated on the role of this uptake mechanism in the regulation of contraction and relaxation of the myocardial cell. A similar ATP-activated calcium uptake mechanism in mitochondria of adrenal medullary chromaffin cells was described (168), which was inhibited by azide, oligomycin, and 2,4-dinitrophenol.

The adrenal medullary chromaffin granules may also play a role in the termination of an evoked secretory response. The fact that calcium readily penetrates the chromaffin granule during stimulation (160), coupled with the demonstration that the ATP-Mg$^{+2}$-dependent pump in the granule has many properties of the calcium pump in muscle (192), would indicate that the chromaffin granule may act as an active sequestrating organelle for calcium. The adrenal chromaffin granules have also been shown to take up calcium during catecholamine synthesis, since stimulation of the adrenal glands with acetylcholine in the presence of 3,4-dihydroxyphenylalanine (dopa) and [45]Ca resulted in a greater accumulation of [45]Ca in the granule fraction than stimulation in the absence of dopa (174). Furthermore, a relatively large amount of the intragranular calcium was found to be held in a nondiffusible form and was considered part of the binding complex for catecholamines in the adrenal medulla (174). It is possible, therefore, that uptake of calcium into the chromaffin granule may serve the dual purpose of terminating the secretory response (81) and of participating in the synthesis and storage of new catecholamines (174) to replenish the granules following an evoked secretion of their contents.

Termination of the secretory response or of muscle contraction could conceivably also be brought about by an active pumping of calcium out of the cell against a concentration gradient. This could be achieved by means of the outwardly directed membrane sodium–calcium pump, similar to the one described by Blaustein and Hodgkin (199) and by Reuter and Seitz (200). An indirect confirmation of such a mechanism derives from the finding that ouabain, which blocks the membrane sodium–calcium pump and increases the concentration of intracellular calcium, enhances both spontaneous and carbachol-induced catecholamine secretion from the adrenal medulla (154) and exhibits positive inotropic effects on the myocardium.

The described mechanisms for the termination of the secretory response of the adrenal medulla received further confirmation from Borowitz (167) who showed that the secretory response to acetylcholine, which is normally terminated in a few seconds after the removal of the stimulus, is prolonged under conditions that promote an influx of large amounts of calcium into the chromaffin cell—such as high concentration of extracellular calcium. The slow removal (either by intracellular binding or by efflux) of the relatively large amounts of calcium from the adrenal medullary cells may account for the prolonged catecholamine release under these conditions (167). Local anesthetics block the influx of calcium as well as the secretory response of the adrenal medulla (57, 161, 167) and also inhibit contractility and associated calcium movements in both striated and smooth muscles (201, 202).

Drugs and other factors that release intracellular calcium or block its uptake into intracellular organelles have profound effects on muscle by initiating contraction or delaying relaxation (50, 203). In an analogous sense, it would be expected that agents or factors that increase the intracellular calcium-ion concentration in the adrenal medullary chromaffin cell could initiate the release of catecholamines or, alternatively, potentiate or prolong an evoked secretory response. From the close parallelism between muscle and the adrenal medulla with respect to calcium binding, as already described, it might be predicted that drugs or factors that affect muscle function by influencing calcium mobility would have the same type of effect on adrenal medullary secretion: catecholamine release would be analogous to muscle contraction, and termination of the secretory response would correspond to relaxation (168). Indeed, agents such as caffeine (56, 57), barium, thiocyanate, sulfhydryl reagents, and ouabain (145, 168), all of which increase the level of free calcium in the cytoplasm by influencing intracellular calcium movements and binding, have been shown to potentiate or evoke adrenal medullary secretion and also to contract muscle (145). Furthermore, anoxia, nitrogen, and cyanide (190) increase spontaneous or evoked catecholamine release from the adrenal medulla, and cooling enhances the secretion of vasopressin from the neurohypophysis in the absence of extracellular calcium (186). These observations could be related to a diminished mitochondrial or microsomal calcium uptake (168)—mechanisms that may well be responsible for the termination of the secretory response (145, 168).

## PHARMACOLOGICAL MECHANISMS OF STIMULUS–SECRETION COUPLING IN ADRENAL MEDULLA

Somlyo and Somlyo (203) broadly classified the mechanisms of smooth muscle contraction into "electromechanical coupling" (regulation of the contractile system by changes in the membrane potential, action potential frequency, or both) and "pharmacomechanical coupling" (the action of compounds on the contractile system independent of the membrane potential and action potential). Evidence that similar mechanisms apply to skeletal and cardiac muscle is extensive (50, 198, 204). Since, as previously discussed, similar molecular mechanisms appear to underlie the processes of excitation–contraction coupling in muscle and stimulus-secretion coupling in the adrenal medulla and other secretory cells (78, 81, 145, 168), a similar classification of the mechanisms of stimulus-secretion coupling was proposed by Rahwan et al. (57). The terms electromechanical coupling and pharmacomechanical coupling could justifiably be retained for this purpose, since there is mounting evidence that the process of secretion may indeed involve a contractile mechanism² (145, 146). The possible subcellular mechanisms by which drugs may evoke secretion are as follows:

A. Electromechanical coupling (dependent upon extracellular calcium): membrane depolarization, leading to increased permeability to inorganic ions, and an influx of extracellular calcium into the secretory cell
B. Pharmacomechanical coupling (dependent upon intracellular calcium):
  1. Release of calcium from the endoplasmic reticulum
  2. Inhibition of calcium uptake by the endoplasmic reticulum
  3. Release of calcium from mitochondria
  4. Inhibition of calcium uptake by mitochondria
  5. Release of calcium from cell membrane storage sites
  6. Inhibition of calcium binding by the cell membrane
  7. Inhibition of calcium efflux from the cell by inhibition of the membrane sodium–calcium pump
C. Mechanisms independent of extracellular or intracellular calcium:
  1. Direct interaction with intracellular contractile proteins
  2. Inhibition of hypothetical "inhibitory factor" (troponin?)
  3. Interference with the intragranular or intratubular storage mechanism
  4. Displacement of secretory product from intragranular or intratubular storage by false transmitter-type effect
D. Physical damage to cell membranes or membranes of intracellular organelles of the secretory cell, leading to passive diffusion of calcium and/or secretory product

It is conceivable that secretagogues like acetylcholine, nicotine, carbachol, pilocarpine, potassium, hista-

mine, serotonin, bradykinin, and angiotensin I evoke catecholamine secretion from the adrenal medullary cell by electromechanical coupling (57), involving depolarization of the chromaffin cell membrane, with a dependency on extracellular calcium (26, 27, 150, 155, 156). On the other hand, drugs that evoke catecholamine secretion from the adrenal medulla in the absence of extracellular calcium [e.g., caffeine, aminophylline, chlorpromazine, dextroamphetamine, reserpine, tyramine, phenylethylamine, barium, strontium, thiocyanate, and acetaldehyde (33, 55, 57, 58, 152, 163, 168, 169)] could conceivably exert their secretory effect by pharmacomechanical coupling (57), with a dependency on intracellular calcium.

In addition to electromechanical and pharmacomechanical coupling, other alternative mechanisms for drug-induced catecholamine secretion from the adrenal medulla should be considered, such as interference with the catecholamine storage mechanism (independent of both extracellular and intracellular calcium) or physical damage to biological membranes. For example, acetaldehyde releases catecholamines from the adrenal medulla presumably by a direct interaction between the aldehyde and the chromaffin granule protein, resulting in a possible alteration of the chromaffin granule membrane structure (55), or by an interaction between the aldehyde and the catecholamines forming the tetrahydroisoquinoline derivatives which act as "false transmitters" (205). Both interpretations are consistent with the finding of Schneider (55) that acetaldehyde-induced catecholamine secretion from the adrenal gland and from isolated chromaffin granules, which occurs in the absence of calcium in the perfusion or incubation medium, is not accompanied by the release of intragranular or cytoplasmic proteins. Rahwan (146) and Rahwan et al.[2] (57) reported that chlorpromazine evoked catecholamine secretion from the adrenal medulla by physical disruption of, and mobilization of calcium from, the mitochondria of the chromaffin cells. Chlorpromazine-induced mitochondrial structural damage in the adrenal medulla was also reported by Mäkelä and Vapaatalo (206). It is conceivable that the reserpine-induced catecholamine release from the adrenal medulla, which occurs in the absence of extracellular calcium (33, 152), may represent a calcium-independent mechanism based on interference with the intragranular storage mechanism (30). Tyramine may also release adrenal catecholamines by a mechanism that does not require calcium (152), analogous possibly to its ability to displace norepinephrine from storage granules in adrenergic nerve endings (207). This is supported by the finding that tyramine-induced adrenal catecholamine secretion is not accompanied by release of intragranular protein or dopamine-β-hydroxylase (58). Similar results were reported by Schneider (58) for the effect of l-amphetamine on the adrenal medulla. Likewise, the initial phase of the secretory effect of dextroamphetamine on the adrenal medulla was reported by Rahwan et al. (57) to be calcium independent and to involve a tyramine-like displacement of catecholamines from their storage sites into the cytoplasm from where the amines could be recovered.

It is likely that pharmacomechanical mechanisms of secretion may represent the primary means by which some drugs evoke secretion even in the presence of extracellular calcium (57). It is also conceivable that electromechanical and pharmacomechanical mechanisms may be operative simultaneously under physiological conditions where calcium is present in the cellular environment.

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