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STIMULUS-SECRETION COUPLING *in* CHROMAFFIN CELLS

Volume I

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Stimulus-Secretion Coupling in Chromaffin Cells

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INTRODUCTION

The adrenal chromaffin cell occupies a unique place in modern cell and neurobiology. Originating from the neural crest, chromaffin cells are believed to be modified postganglionic sympathetic neurons. Thus, over the past two decades, they have served as one of the most readily available model systems in studying the mechanism(s) of neurotransmitter release. The pioneering experiments of W. W. Douglas and his colleagues in adrenal chromaffin cells have firmly established the now widely accepted concept of calcium-dependent stimulus-secretion coupling as a universal mechanism for the exocytotic secretion of a variety of neurotransmitters and hormones, packaged in intracellular storage organelles of many different cell types.

Over the years, a number of excellent reviews on chromaffin cells have been published, most of them dealing with general aspects of chromaffin cell biology. However, during our own involvement in the study of the mechanism of catecholamine secretion, we felt the need for a more specialized treatise on what we believe the be the central *raison de etre* of chromaffin cells: catecholamines and neuropeptides, synthesized and stored in the cells, are released in a strictly controlled fashion and upon special physiological demand only. So what then are those mechanisms that translate the extracellular signals into the cellular responses, culminating in the secretion of storage products? What do we really know about the intracellular mechanism linking cell activation to secretion?

In order to address these questions in as broad a fashion as possible, we have gathered for these volumes authoritative contributions of leading experts on the adrenal chromaffin cell, and in particular, on the mechanism(s) of stimulus-secretion coupling. As it is unavoidable in multiauthored monographs like this, some of the contributions contain several seemingly redundant treatises of similar issues; however, we deliberately invited such complementary chapters, since every author is presenting his individual concept of a very complex issue.

As is evident from studying the various chapters, we are still far from being able to draw a unified picture of what might be going on during the processes of stimulus-secretion coupling in the adrenal chromaffin cell. On the contrary, the more detailed our information becomes about the intracellular events following stimulation of the cells and leading up to the exocytotic secretion, the more emerges our obvious lack in understanding of the finer tunings of the cell biology of the chromaffin cells. At present, exciting new developments are rapidly changing some of our basic understanding about, for example, membrane biophysics of ion channels, intracellular second and third messengers, the temporal and spatial role of calcium homeostasis, or the role of cytosolic proteins in mediating exocytotic membrane fusion, to name but a few of the unresolved issues. Furthermore, with more details emerging on the biological, biophysical, and molecular biological patterns of stimulus-secretion coupling in chromaffin cells, the more the individuality of this particular cell will become evident.

In editing this book, we therefore attempted to provide, for the first time, an in-depth resume of several aspects of our current (as of 1986) understanding of stimulus-secretion coupling. The authors were asked not only to provide a state-of-the-art review in their respective fields of interest, but also to look ahead and try to address unresolved issues and those relevant questions to be tackled in the near future. Thus, this monograph, centered around a key function in the cellular biology of adrenal chromaffin cells, combines solid evidence on what is presently known as well as more speculative individual assessments as to future developments. Concomitantly, however, this book is published with the cautionary notion on extrapolating our current knowledge about chromaffin cells to apparently similar mechanisms governing stimulus-secretion coupling in other secretory cells or to different endocrine organs.

For technical reasons the 15 chapters have been arranged in two volumes. Nevertheless, these two volumes are intended as a contiguous, single monograph on the multifaceted issue termed ''stimulus-secretion coupling''.

In the first chapter, S. W. Carmichael presents a detailed description of the anatomical morphology of the adrenal medulla. J. H. Phillips deals in two chapters with an extensive discussion of chromaffin granules: beyond the biogenesis of these storage organelles, their structure, and their dynamics, the author discusses the fate of chromaffin granules during the perpetual cycles of exocytosis and endocytosis.

More recently, a number of bioactive peptides, in particular enkephalins, were found to be localized in chromaffin granules and co-released together with the catecholamines; these exciting new findings are summarized by C. D. Unsworth and O. H. Viveros in Chapter 4.

Calcium is generally believed to be of central importance for a number of biochemical processes linking cell stimulation to exocytotic secretion. One of the unresolved issues relates to the question of calcium buffering and calcium homeostasis in chromaffin cells and the possible role of the granules in these processes. In Chapter 5, Gratzl discusses possible pathways for the uptake of calcium into chromaffin granules and the relevance of Na^+/Ca^{2+} exchange across the granules for intracellular calcium homeostasis.

Specific calcium-regulating and -regulated cytosolic proteins, which are believed to be involved in intracellular signal transduction, are discussed in the next group of chapters. As described in Chapter 6, Trifaró and Kenigsberg used classical pharmacological approaches as well the fusion of antibody loaded erythrocyte ghosts with cultured chromaffin cells to probe the central role for calmodulin in stimulus-secretion coupling. The relevance of cytoskeletal proteins in intracellular signal transduction, especially the structural and functional role of a spectrin-related, actin-associated protein α-fodrin is reviewed by Aunis, Perrin, and Langley in Chapter 7. In recent years, a number of cytosolic proteins, such as the phospholipases, proteinkinases, etc., have been proposed to mediate calcium action during exocytosis. In Chapter 8, Pollard and his colleagues primarily discuss the family of synexins and immunologically related proteins, which seem to regulate membrane contact and fusion in a calcium-dependent fashion. P. I. Lelkes describes (Chapter 9) how liposomal vectors can be employed to introduce bioactive (macro) molecules, such as cytoskeletal proteins, etc., into intact chromaffin cells to study their involvement in the cascades of stimulus-secretion coupling.

Recognition of the stimulus at the plasma membrane is the primary event in activating a cellular response. Yet, as summarized by Rosenheck in Chapter 10, our present knowledge of the functional biochemistry at the plasma membrane level is quite limited, presumably due to too scarce a usage of isolated chromaffin cell plasma membrane preparations.

Adrenal chromaffin cells are activated via nicotinic and/or muscarinic receptors, depending on the species and probably also on physiological idiosyncrasies. The importance of muscarinic stimulation has recently been emphasized due to the clear linkage of muscarinic activation to the phosphoinositide metabolism in a number of eukaryotic cells. In Chapter 11, Allan Schneider discusses muscarinic receptor mechanisms in adrenal chromaffin cells.

Cell activation comprises transmembranal ion fluxes. In intact cells, the various ion channels of the plasma membrane have been characterized and the ion fluxes pertaining to the intracellular signal transduction have been widely studied. In Chapter 12, Kirshner presents an overview of the more classical biochemistry and electrophysiology of calcium and sodium channels in intact chromaffin cells. However, over the past few years, electrophysiology has been revolutionized by analyzing single channels using the patch-clamp technique. Thus, Kidokoro summarizes in Chapter 13 these more recent developments in membrane biophysics of chromaffin cells.

Inhibitory modulations of the secretory response, often termed desensitization, are discussed in the final two chapters. In Chapter 14, Garcia and his co-workers review the

evidence for the pivotal role of calcium channel activation for the onset of stimulus-secretion coupling and the inactivation of the secretory response by sustained elevated intracellular calcium concentrations. Finally, in Chapter 15, Bruce Livett summarizes our current understanding of various parameters, in particular the role of neuropeptides, which in vitro and in vivo are involved in the modulation of the secretory response in adrenal chromaffin cells.

During the entire process of compiling and editing this monograph, we were encouraged by Marsha Baker, the senior editor in charge of the Uniscience Series at CRC Press, and guided by the helpful advice and patience of our coordinating editor, Anita Hetzler. We especially appreciate the enduring understanding and support from our families, who for the many years of our close collaboration have tolerated our time-consuming fascination with the chromaffin cells more or less patiently. We therefore dedicate this book to our families, especially to our wives, Alma Rosenheck and Iris Lelkes.

Rehovoth and Bethesda, December 1986

Kurt Rosenheck
Peter I. Lelkes

THE EDITORS

Kurt Rosenheck received his Ph.D. in Physical Chemistry from the Hebrew University of Jerusalem in 1959 for work on polyelectrolytes, under the direction of Aharon Katzir-Katchalsky. He then was a research assistant in the Polymer Department of the Weizmann Institute of Science, Rehovot, Israel, and subsequently worked as a research fellow with Paul Doty in the Chemistry Department of Harvard University.

He spent 1969 as a resident scientist in the Neuroscience Research Program of the Massachusetts Institute of Technology, Cambridge, directed by Francis O. Schmitt. In 1975 he was a visiting investigator in the Endocrinology Section, directed by Martin Sonenberg, of the New York Sloan-Kettering Institute for Cancer Research. In 1977 he was appointed Associate Professor at the Weizmann Institute's Department of Membrane Research. During the years 1984 and 1986 he held Visiting Professorships at the Universities of Constance and Bielefeld, in West Germany, as well as at the University of Berne in Switzerland.

Starting out as a polymer spectroscopist, he later applied ultraviolet light spectroscopic techniques to the study of protein conformation and lipid-protein interactions in biological membranes. This led to his interest in the membrane-linked events occurring during the stimulus-secretion response, a field he has been active in for the last 10 years.

Peter I. Lelkes, born in 1949 in Budapest, Hungary, received his training in physics, biochemistry, cell biology, and membrane biophysics at the Technical University in Aachen, West Germany. In 1977 he joined the Department of Membrane Research at the Weizmann Institute of Science in Rehovoth, Israel, as a postdoctoral fellow to work on physicochemical aspects of protein-lipid interactions of biological membranes. Subsequently, as a staff scientist in the same department, he focused his interest on the mechanism of membrane fusion of biological membranes, and in particular, on the involvement of cytosolic proteins in the stimulus-secretion coupling in chromaffin cells and other secretory systems.

In 1983, he went to the National Institutes of Health, Bethesda, Md., to further study the pathways of intracellular signal transduction of secretory cells. Working in the National Institute of Diabetes, Digestive, and Kidney Diseases, he currently holds the position of Visiting Scientist in the Laboratory of Cell Biology and Genetics, and continues to study cell biological aspects of the activation of adrenal medullary chromaffin and other endocrine cells.

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Chapter 1

MORPHOLOGY AND INNERVATION OF THE ADRENAL MEDULLA

Stephen W. Carmichael

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I. INTRODUCTION

The chromaffin cells of the adrenal medulla have proven to be excellent models for the study of stimulus-secretion coupling. They are secretory cells that behave like neurons in many important respects. They are widely considered to be modified postganglionic sympathetic neurons, but differ from typical sympathetic cells in two important respects: they generally secrete epinephrine rather than norepinephrine as their transmitter, and they do not have processes (dendrites or an axon) characteristic of neurons. Both of these differences are apparently related to the proximity of the adrenal medulla to the adrenal cortex.

The adrenal medulla is typically the central portion of the adrenal gland. Adrenal means adjacent to the kidney (Latin, ad = to and ren = kidney) and medulla means the core (Latin, marrow). While the term adrenal medulla is widely used and recognized, the technically proper term (nomina anatomica) is glandula suprarenalis medulla. The suprarenal gland is properly referred to in the human where the gland is above (supra) the kidney. In most quadripeds, the gland is anterior or medial to the kidney and can be called the adrenal gland. In lower vertebrates, such as fish and reptiles, the glandular tissue lies among or between the kidney tissue and is called the interrenal gland. For the sake of simplicity and clarity, in this review the organ will be consistently referred to by its most common name, the adrenal medulla.

The adrenal medulla was apparently first mentioned in 1611 by Caspar Bartholinus, who described the adrenal glands as containing a cavity full of "black bile" (Figure 1). The concept of the adrenal gland as a hollow organ persisted for two centuries, even though there were numerous unsuccessful attempts to find a duct leading from this alleged vesicle. Credit is often given to Georges Cuvier for describing the adrenal gland as consisting of a cortex and a solid medulla in 1805, although the terms cortex and medulla were apparently first introduced by Huschke in 1845. It was later recognized that the fluid-filled cavity described previously was due to rapid post-mortem disintegration of the medulla.

In 1856 Alfred Vulpian described an unusual staining reaction of the adrenal medulla. He found that this portion of the adrenal gland developed a green color when treated with ferric chloride. Furthermore, secretions from the adrenal gave a similar reaction. Apparently, there was something unique in this part of the adrenal gland and in its secretions which reduced ferric chloride to ferrous chloride. Jacob Henle, in 1865, found that salts of chromium gave a characteristic color reaction with the adrenal medulla. At about this same time, Albert von Kölliker published a detailed account of the histology of the adrenal gland. He concluded that the cortex and medulla of the adrenal were structurally and functionally distinct. Since the medulla contained an extremely abundant nerve supply, Kölliker regarded this portion of the gland as being related to the nervous system. In retrospect, it is fair to say that Kölliker was a visionary in making the conclusions that he did, particularly in view of the fact that the technology of preparing tissues for examination and microscopes of his time was primitive by today's standards. In 1902, Kohn[93] published his classical account of the organs of the body that stained with chromium salts. He coined the phrase "chromaffin cells" to describe the cells which reacted in this manner. This terminology has persisted to the present day, even though there have been attempts to change it. By early in this century, through the efforts of the scientists mentioned above and many others, the structure of the adrenal medulla at the light microscopic level was well understood.

The next breakthrough in morphological descriptions of the adrenal medulla had to await the introduction of the electron microscope to biological studies. Hillarp et al.[79] published electron micrographs of crushed adrenal medullary cells in 1954. Lever[99] was the first to study the adrenal medulla with the electron microscope. He demonstrated electron-dense membrane-bound vesicles as a predominant feature of the chromaffin cell cytoplasm using potassium dichromate and osmic acid as a fixative. Sjöstrand and Wetzstein[129] were the first

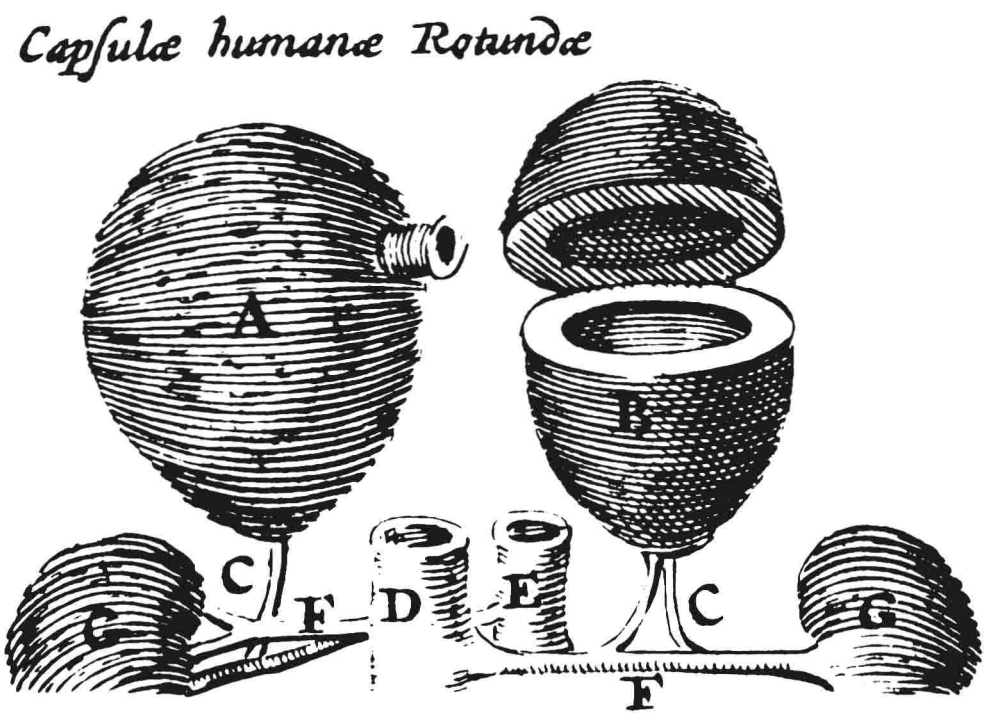

FIGURE 1. This is a copy of an original woodcut published by Caspar Bartholinus in 1611. The right and left adrenal glands are depicted as A and B, respectively, and the kidneys are labeled G. Note that the left adrenal is a hollow sphere. The concept that the adrenal gland is a vesicle filled with "black bile" held sway for almost 200 years.

to call these vesicles "chromaffin granules". Numerous studies have been published since then and will be discussed in the section on ultrastructure.

Studies on the embryology of the adrenal gland were done at about the same time as the definitive light microscopic studies, probably due to the instrumentation of that era. In 1831, Arnold stated that the adrenals developed from the mesonephros (Wolffian body). In 1852, Henry Gray made a distinction between the adrenal glands and the mesonephros by pointing out that the adrenals arose between the mesonephros and the aorta. Remak published papers in 1847 and 1855 which indicated that the adrenals were embryonically related to the sympathetic ganglia. During the next 100 years there was a large number of descriptions which did little to improve on these early observations.

The definitive studies on the embryology of the adrenal medulla were made in quail-chick chimeras by LeDouarin and Teillet.[98,135] In these studies, precursor cells were transferred from the neural crest of the quail to chick and vice versa. The final differentiated cells could be distinguished by differences in the staining of the nucleus of the quail-chick cells. It was found that when the graft covered the length of the neural primordium between the levels of somites 18 to 24, the entire supply of chromaffin cells of the adrenal medulla was included. In contrast, the graft did not participate in the formation of the adrenal medulla when it was inserted either anteriorly or posteriorly to this area. The neural crest derivatives in the cervicothoracic region are restricted to the sensory and sympathetic chain ganglia, the aortic and adrenal plexuses, and the adrenal medulla. Therefore, as suggested by Remak in 1847, the adrenal medulla does develop from the same precursors as the sympathetic ganglia.

A mammalian model for the development of the adrenal medulla can be found in the opossum. In this remarkable animal, newborns do not possess an adrenal medulla, but rather,

the precursor cells differentiate and form the adrenal medulla during the first 4 days after birth.[36] This marsupial may present a particularly good mammalian model for future experimental studies on the development of the adrenal medulla.

II. GROSS ANATOMY

The mammalian pattern is quite consistent, so that a description of the gross anatomy of the adrenal gland in one mammal is generally applicable to others. As an example of a mammal of general interest, the gross anatomy of the human adrenal gland will be described.

The adrenal (suprarenal) gland, as the name implies, is located at the superior pole of each kidney. The right gland is pyramidal in shape, whereas the left gland is semilunar in shape and tends to be somewhat larger. Kreiner[96] described the shape and weight of the human adrenal medulla in various age groups. In the newborn, the medulla accounts for less than 1% of the total volume of the adrenal gland, and by adulthood it occupies 9% of total adrenal volume. In the adult the adrenal medulla has an average weight of 0.43 g.

Both adrenal glands are enclosed within a condensation of extraperitoneal connective tissue which is called the renal fascia. As this membranous layer also encloses the corresponding kidney, it is important to realize that there is a relatively distinct layer of fascia between the kidney and the adrenal gland so that the adrenal gland is completely enclosed. The renal fascia is connected to the diaphragm above so that the position of the adrenal glands does not depend on the position of the kidneys. Therefore, in cases of congenital absence, ectopia, or ptosis of the kidneys, the adrenal glands are almost always in their normal location.

The arteries to the adrenal glands are numerous, since the glands are highly vascular organs. The superior adrenal arteries may number from 3 to 30 and are typically derived from the inferior phrenic artery. The middle adrenal artery typically arises as a single branch directly from the aorta above the origin of the renal artery. The inferior adrenal artery typically arises from the renal artery. Additional arteries may be derived from a ureteric artery, gonadal artery, or other arteries in the vicinity. While the human pattern resembles the general mammalian pattern, there are some significant variations.[44]

The main venous drainage is via the adrenal vein, which arises directly from the central vein within the gland. The adrenal vein emerges from the hilus of the gland and usually empties into the left renal vein on the left and directly into the inferior vena cava on the right. Variations in the venous drainage were described by Khalique and Monkhouse.[89] The left adrenal vein was almost always (96%) joined by the left inferior phrenic vein, and this adrenophrenic trunk drained into the left renal vein. The right adrenal vein was duplicated or triplicated in 40% of the cases, although this was not seen on the left. In more than 20% of specimens, there was substantial drainage from the posterior aspect of the right adrenal gland that is connected with the hemiazygos vein, thereby discharging at least some of the secretory product to the heart via the superior vena cava.

A matter of continuing concern is the manner in which blood is circulated within the adrenal gland. Specifically, several studies have shown that there is not a portal system between the cortex and medulla of the gland. Extensive studies at the light microscopic level[46] and with the scanning electron microscope[90] showed that capillaries within the medulla originate directly from medullary arteries and drain through deep venous radicles into the tributaries of the central vein. The cortex is drained through separate peripheral venous radicles which flow into the tributaries of the central vein. Therefore, most of the cortical blood, rich in glucocorticoids, flows in the medulla exclusively through the radicles of the central vein and not through the medullary capillary plexus.

The lymphatics of the adrenal medulla have been studied by scanning electron microscopy (SEM).[7] This study in the bull showed that the lymphatics are rather extensive. A role for

lymphatics in the circulation of secretory products from the adrenal medulla has not been established.

At the gross anatomical level, the nerves to the adrenal glands are numerous and are derived from the celiac and renal plexuses. They form a plexus on the surface of the adrenal gland and traverse the cortex to end mainly on cells in the medulla. Small ganglion cells may also be found within the medulla. Details of this innervation and how it fits into the pattern of the sympathetic nervous system will be discussed below.

While these patterns for the anatomy, blood supply, and innervation of the adrenal medulla conform well to mammals, there are considerable differences in lower animals. In the higher mammals, including primates, the gross features of the adrenal gland and the adrenal medulla are quite similar. There are some minor differences, such as an abundance of chromaffin cells, in the rabbit. Another notable difference is in the two-toed sloth, where the adrenal gland is not associated with the kidney.[158] This is due to the fact that the kidney lies in the pelvis in this animal, leaving the adrenal gland in the abdomen as a cylindrical organ lying alongside the aorta and inferior vena cava. In this, as in other mammals, the medulla is completely surrounded by the cortex.

As reviewed by Coupland,[41] there are some significant variations in lower mammals. Specifically, in monotremes such as the spiny anteater and duck-billed platypus, the medullary cells tend to be arranged somewhat differently with respect to the cortex. For example, chromaffin cells are concentrated at the caudal pole of each gland in the spiny anteater.

In other vertebrates such as amphibians, reptiles, and birds, the chromaffin tissue is distributed throughout the adrenal glands, intermingling with cortical cells. The chromaffin cells may be distributed singly, in groups, or in cords among the cortical elements. In these animals the adrenal gland is more properly referred to as the interrenal. Since these animals are characterized by internal gonads that tend to be abdominal in position, the interrenal glands are usually in close proximity to these organs, as well as being related to the ventral aspect of the elongated kidney.

Amphibians are the lowest class of vertebrates in which the adrenal chromaffin cells have an intimate relationship with the steroid-secreting cells commonly referred to as cortical cells. This relationship does not exist in animals below amphibia on the phylogenetic scale. For example, in bony fish (teleosts) the chromaffin cells are scattered diffusely along the posterior cardinal veins. The cells may appear singly or in groups of varying sizes and shapes. Some chromaffin cells have an intimate relationship with the most rostral portion of the kidney, which is referred to as the head kidney. The steroid-secreting organ, referred to as the interrenal gland, is separate in these animals. A similar pattern is seen in cartilaginous fish (elasmobranchs). In cyclostomes the chromaffin cells are even more scattered, being found within the walls of various organs such as the heart and kidney. No chromaffin tissue has been identified in amphioxus, which is considered to be the most primitive vertebrate.

There are reports in the older literature describing catecholamine-containing cells in the nervous system of a variety of invertebrates. These chromaffin cells are located mostly within the nerve ganglia. In a nematode *(Caenorhabditis elegans),* where all 302 cells of the nervous system have been fully characterized, there are some dopamine-synthesizing cells, but nothing that corresponds to an adrenal medulla.[159] For a more complete review of the older literature on the comparative anatomy of the adrenal medulla with numerous references, the reader is referred to works by Coupland[41,44] and Varano.[143]

III. LIGHT MICROSCOPY

Although the distribution of intra- and extraadrenal chromaffin cells varies among species, the appearance of these cells in the light microscope is rather uniform. Chromaffin cells are relatively large and irregular in shape. Within the typical mammalian adrenal medulla, they

appear to conform to the available space so that they are columnar or polygonal in shape. Isolated chromaffin cells tend to assume a spherical or ovoid shape with a diameter of about 20 μm. This corresponds well to an estimate of cell size within the rat adrenal medulla.[109] The nucleus is about 5 μm in diameter. A characteristic feature of these cells is the granular appearance of the cytoplasm. These cytoplasmic granules characteristically are stained brown when oxidized by potassium dichromate: this is the basis of the chromaffin reaction for which the cells and granules are named. The granules also demonstrate a characteristic color reaction when treated with other oxidizing agents. For example, they become green with ferric chloride, yellow with iodine, and brown with osmium tetroxide. This latter reaction was observed in 1918 by Cramer.[49] He referred to "adrenalin granules" as "giving the appearances of fine coal dust scattered over the medulla". He also observed that these osmiophilic granules passed into the blood vessels during an increase in secretory activity.

Although adrenal chromaffin cells have many similarities to postganglionic sympathetic neurons, they clearly vary from neurons in their shape. The absence of neuronal processes in adrenal chromaffin cells is probably due to the influence of steroids secreted by the surrounding adrenal cortex. When chromaffin cells are isolated and grown in culture, they extend processes and form functional cholinergic synapses, which indicates that they have the potential to be shaped and behave like typical neurons.[110] When steroids are added to the culture medium, the extension of processes is inhibited.[136,142] Thus, in vivo, the cortical steroids may be partially responsible for the shape of adrenal chromaffin cells. In a related fashion, the steroids may be responsible for epinephrine (rather than norepinephrine) being the main neurohumor of the adrenal medulla, since steroids have a positive influence on the conversion of norepinephrine to epinephrine.[38]

At the light microscopic level, two distinct populations of chromaffin cells have been identified in the adrenal medulla. In 1953, Hillarp and Hökfelt[78] introduced the iodate method to differentiate epinephrine- from norepinephrine-containing cells. Eränkö[60] showed that the epinephrine- and norepinephrine-containing cells could be differentiated by fluorescence methods. Since these early studies, there have been several techniques devised to unequivocally demonstrate that epinephrine and norepinephrine are stored in separate cells.[84] The proportion of these cells to each other and their distribution within the adrenal medulla varies among species and with the age of the animal. In several mammals, including the hamster, the norepinephrine-containing cells are located at the periphery of the medulla.[113] This is an interesting paradox, since one would think that the influence of cortical steroids would be strongest in this region and the synthesis of epinephrine would be favored.

In addition to chromaffin cells, the adrenal medulla of mammals has been observed to contain some ganglion cells. While these cells are thought to play a part in the nervous system relay, the actual position of these cells within the relay and their function are not known.

Within the mammalian adrenal medulla the cells are arranged in groups or irregular cords around blood vessels. This arrangement, referred to as a tunneled muralium, has been confirmed by SEM.[37]

IV. ULTRASTRUCTURE

The predominant ultrastructural feature of chromaffin cells are numerous membrane-bound vesicles (Figure 2). These are often called "chromaffin granules", a term that refers back to the image seen in the light microscope. However, it is the interpretation of several workers that these membrane-bound structures are more properly referred to as "chromaffin vesicles". Since the organelles were isolated in 1953,[23,80] they have been well characterized by biochemical studies.[150] Perhaps because chromaffin vesicles were the first secretory organelle to be isolated, they are the best characterized.

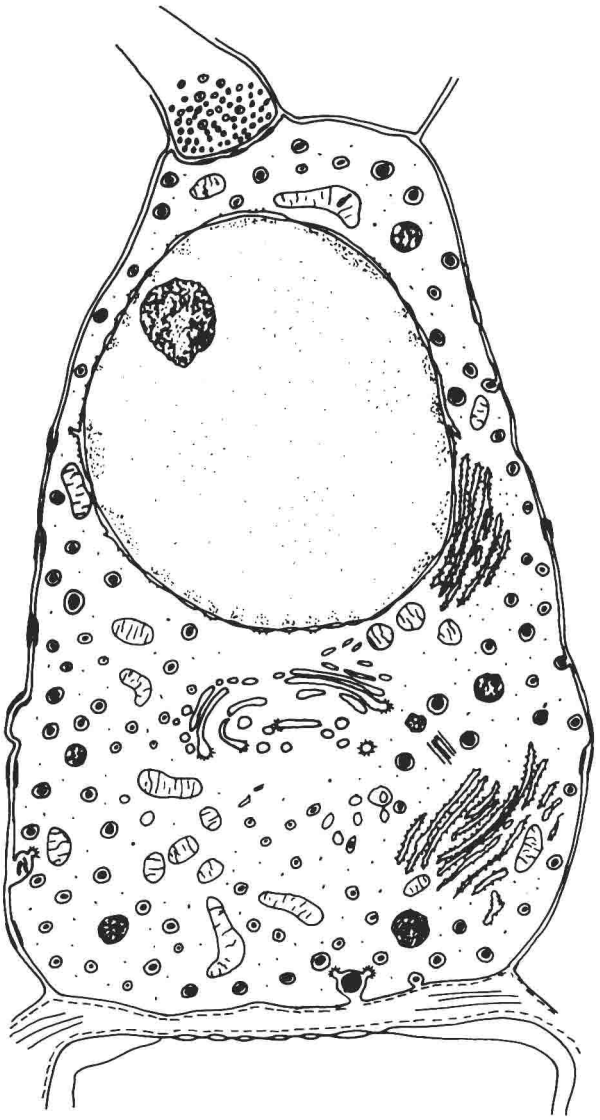

FIGURE 2. This is a schematic drawing of an adrenal chromaffin cell illustrating the typical ultrastructural features. The membrane-bound, dense-cored vesicles are chromaffin vesicles containing norepinephrine. Epinephrine-containing vesicles, which are not seen in this illustration, would be less dense, and the halo around the core would be less conspicuous. A nerve terminal is illustrated at the top, and a fenestrated endothelial cell is at the bottom. Modified from Coupland[44] and Grynszpan-Winograd.[69]

Regrading chromaffin cells, a number of studies have been done which include quantitative information on the size of chromaffin vesicles, and these studies have been tabulated in Table 1. This table also points out the number of species in which the chromaffin cells of the adrenal medulla have been studied with the electron microscope. Although a variety of animals have been studied, one is impressed by the similarity of the morphology of these cells at the ultrastructural level. The mammalian adrenal chromaffin cell will be described, but it is essentially the same for chromaffin cells of other animals.