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SYNTHESIS, STORAGE AND SECRETION OF ADRENAL CATECHOLAMINES

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PREFACE

Since the memorial observation by Oliver and Schäfer (1895) that suprarenal extracts contained cardioactive pressor substance, adrenal medulla have been the central subject in endocrinology. A variety of physiological concepts in cellular and synaptic events have been much developed from this organ. The role of catecholamines spread out to a number of autonomically innervated tissues and central nervous systems as well. Today, obviously, the adrenal medullary cells are the most distinctve prototype for neuronal and neurosecretory cells.

The major pathways for the synthesis and metabolism of catecholamines were fully understood in cellular, subcellular and molecular levels. Substantial recognition of catecholamines has been made very clearly. At the same time, recent developments in biochemical, physiological and pharmacological techniques have enabled investigation of membrane receptors linked to the action of catecholamines. However, when we retrospect in the primary function of adrenal medulla, - the synthesis, storage and secretion of catecholamines-, we will realize the fact that functional recognitions of this organ are so rigid and fragmental.

Adrenal medullary cells are neural crest origin and their functions are wholly under the control of first order preganglionic fibers. Each function could be well studied in these cells, since they show the highest activities of catecholamine synthesizing enzymes, have the well developed catecholamine storing granules and readily secrete their contents upon stimulation of cholinergic receptor. And in fact, knowledges in separate function have been successfully accumulated to a great amount. But question arises whether the mere summation of each separate function could give the comprehensive figure of the adrenal medullary cells. What is the central event in the receptor-mediated control of cell functions? Is there any integrating mechanism in the functions of adrenal medulla?

It seemed, therefore, to be important to have a meeting for scientists working with different aspects of adrenal catecholamines and to seek for the fundamental and integrating mechanisms which function in adrenal medulla. The 8th International Congress of Pharmacology in Tokyo was an opportune time to have such meeting. The Satellite Symposium of Adrenal Catecholamine followed to the Congress and lasted for three days at Hakone. The proceeding of the meeting are presented in this book. We really hope that all the readers will recognize the present frontier of adrenal catecholamines and realize that adrenal medullary cells are unrivaled one in studies of neuroendocrinology as well as neurotransmitter mechanism.

Such a meeting would not have been possible without the help of many peoples. We are grateful for the help of M.Sandler, H.O.Viveros, E.Costa and C.Gagnon who worked with us on the Programme Committee. We wish to thank the University of Occupational and Environmental Health, Japan, School of Medicine for repeated encouragements. We also wish to thank J. Lavender for his efforts in bringing this book together. Last, but not least, we are grateful for the excellent secretarial help of Y. Toyohira, K. Okuda and K. Hanada.

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LATE PAPER

The Chromaffin Granule: A Challenge in the Past, Present and Future

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ABSTRACT

In this paper we will review the major aspects of research on chromaffin granules: isolation methods, chemical composition and molecular function. Our own recent studies on nucleotide transport and on the morphological substrate of the Mg^{2+}-ATP-ase will also be discussed.

KEYWORDS

Adrenal medulla; chromaffin granules; catecholamines; nucleotide transport; ATP-ase; exocytosis.

INTRODUCTION

In 1953 Blaschko and Welch and Hillarp, Lagerstedt and Nilson reported that centrifugation of homogenates of bovine adrenal medulla led to the sedimentation of the major part of the hormones of this organ. These authors rightly concluded that the medullary hormones, the catecholamines, are stored in subcellular organelles. This classical observation finally led to the isolation and detailed characterization of the catecholamine-storing vesicles of the adrenal medulla, the chromaffin granules.

Twenty-eight years of research on chromaffin granules have yielded numerous publications. In this paper we will review the major challenges which had to be met by the investigators in this field. Due to the limited space only a selected number of references will be given.

ISOLATION OF CHROMAFFIN GRANULES

One of the first problems which had to be tackled was the establishment of isolation methods for these cell organelles. As it can be seen from Table 1 methods employing linear or simplified sucrose density gradients were introduced first. These methods proved essential for the analysis of the composition of chromaffin granules. More recently isoosmotic gradients were described. Chromaffin granules purified by these methods could be incubated afterwards under isotonic conditions which was a prerequisite for various uptake studies.

TABLE 1 The Introduction of Isolation Methods for Chromaffin Granules in the Period from 1953 to 1981

1953
DIFFERENTIAL CENTRIFUGATION: Blaschko and Welch (1953); Hillarp and co-workers (1953)
SUCROSE DENSITY GRADIENT CENTRIFUGATION:
  SEPARATION FROM MITOCHONDRIA: Blaschko and co-workers (1957)
1960
  SEPARATION FROM LYSOSOMES: Smith and Winkler (1966)
  SIMPLIFIED GRADIENT: Smith and Winkler (1967a)
MILLIPORE FILTER: Oka and co-workers (1966)
1970
ISOTONIC GRADIENTS: FICOLL: Trifaró and Dworkind (1970)
  METRIZAMIDE: Morris and Schovanka (1977)
  PERCOLL: Meyer and Burger (1979)
1981

THE COMPOSITION OF CHROMAFFIN GRANULES

After the establishment of isolation methods considerable work was invested to study the composition of chromaffin granules (see Table 2). In the first period the major contributions were made by English and Swedish scientists. However, already in the sixties several new groups joined the field and quite a few of them are still active today. It seems unlikely that future studies will discover new granule components which are important from a quantitative point of view. However, as demonstrated by the recent finding of enkephalins, new components, which are minor in quantity, but have important functional properties, may still be found. Thus most of the protein components of chromaffin granules have not yet been assigned a function.

TABLE 2 Studies on the Composition of Chromaffin Granules in the Period from 1953 to 1981

1953
CATECHOLAMINES: Blaschko and Welch (1953); Hillarp and co-workers (1953)
SOLUBLE PROTEINS: Hillarp (1958b)
Mg^{2+}-ATP-ASE: Hillarp (1958a)
ADP, AMP: Hillarp and Thieme (1959)
PHOSPHOLIPIDS, CHOLESTEROL: Hillarp (1959)
1960
CYTOCHROME b-561: Spiro and Ball (1961); Ichikawa and Yamano (1965)
DOPAMINE ß-HYDROXYLASE: Oka and co-workers (1967)

LYSOLECITHIN: Blaschko and co-workers (1967)

Ca^{2+}: Borowitz, Fuwa and Weiner (1965)

CHROMOGRANIN A: Helle (1966); Smith and Winkler (1967b); Smith and Kirshner (1967)

1970

GTP, UTP: Goetz, Da Prada and Pletscher (1971)

NADH: acceptor oxidoreductase: Flatmark, Terland and Helle (1971)

PHOSPHATIDYLINOSITOLKINASE: Buckley and co-workers (1971)

CHROMOMEMBRIN B: Hörtnagl and co-workers (1971)
(= CYTOCHROME b-561: Apps, Pryde and Phillips, 1980)

MUCOPOLYSACCHARIDES: Fillion, Nosal and Uvnäs (1971); Margolis and Margolis (1973)

ASCORBIC ACID: Terland and Flatmark (1975)

ACTIN: Phillips and Slater (1975)

GLYCOPROTEINS: Eagles and co-workers (1975); Huber and co-workers (1979)

α-ACTININ: Jockusch and co-workers (1977)

SYNAPTIN: Bock and Helle (1977)

GANGLIOSIDES: Geissler and co-workers (1977); Dreyfus and co-workers (1977)

ENKEPHALINS: Lundberg and co-workers (1979); Viveros and co-workers (1979)

1981

MOLECULAR FUNCTION OF CHROMAFFIN GRANULES

Molecular Mechanism of Catecholamine Storage

Since chromaffin granules contain an extremely high concentration of catecholamines the question of the mechanism of storage was already discussed by the first investigators. ATP was soon recognized as a possible partner in catecholamine storage (see Table 3). The claim that the membrane of chromaffin granules was freely permeable to catecholamines at 0°C together with the fact of the stability of the catecholamine store at this temperature led to the postulate of a stable storage complex involving catecholamines and ATP. This seemed finally established when such complexes involving these two components could be demonstrated under in vitro conditions. However, during the last five years this concept has changed. The impermeability of the membranes of chromaffin granules at 0° for small molecules like ions was established. Thus the stability of the catecholamine storage at this temperature could be explained by this property without postulating a storage complex. In fact the absence of a stable storage complex at this temperature was demonstrated by nuclear magnetic resonance studies (see Sen and Sharp, 1981). However, the absence of a stable storage complex does not exclude a certain amount of interaction between the granule components. Indeed, the nuclear magnetic resonance studies indicate that catecholamines and ATP, although free in solution within the granule, interact with each other and possibly also with the acidic chromogranins.

Since chromaffin granules do not contain a stable storage complex of catecholamines additional mechanisms must exist to maintain the high concentration of amines and nucleotides within these organelles.

TABLE 3 Studies on the Molecular Mechanism of Catecholamine Storage in the Period from 1953 to 1981

1953
- ATP: A STORAGE COMPONENT: Hillarp and co-workers (1955); Blaschko and co-workers (1956)
- 0.6 M CONCENTRATION OF CATECHOLAMINES: Hillarp (1959)

1960
- GRANULE MEMBRANE FREELY PERMEABLE TO CATECHOLAMINES: Kirshner, Holloway and Kamin (1966)
- IN VITRO COMPLEXES OF CATECHOLAMINES AND ATP: Berneis, Pletscher and Da Prada (1969)

1970
- GRANULE MEMBRANES: LOW PERMEABILITY AT 0°: Perlman (1976); Johnson and Scarpa (1976)
- NMR-STUDIES: Daniels, Williams and Wright (1978); Sen and co-workers (1979)

1981

Molecular Mechanisms of Catecholamine and Nucleotide Uptake

References for the major breakthroughs in the field are given in Table 4.

TABLE 4 Studies on Catecholamine and Nucleotide Uptake in the Period from 1962 to 1981

1962
- UPTAKE OF CATECHOLAMINES:
  - IN INTACT GRANULES: Kirshner (1962); Carlsson and co-workers (1962)
  - DEPENDENCE ON ATP-ASE: Banks (1965)
  - IN GRANULE GHOSTS: Taugner (1971); Phillips (1974)

1970
  - DRIVEN BY PROTON GRADIENT: Bashford and co-workers (1975)
  - DRIVEN BY Δ pH and $\Delta\psi$: Johnson and Scarpa (1979)
  - RECONSTITUTION IN VITRO: Maron and co-workers (1979)
- ISOLATION OF ATP-ASE: Apps and Glover (1978)
- UPTAKE OF NUCLEOTIDES:
  - CARRIER-MEDIATED TRANSPORT: Kostron and co-workers (1977)
  - DRIVEN BY $\Delta\psi$: Aberer and co-workers (1978); Weber and Winkler (1981)

1981

The uptake of both catecholamines and nucleotides is driven by a proton-gradient maintained by the proton pumping ATPase of the granule membrane. This enzyme, which has been isolated, was shown to be very similar to the proton pumping ATPase of mitochondria (Apps and Schatz, 1979). Based on these data we calculated (Winkler and Westhead, 1980) that a single granule should possess about 10 molecules of this enzyme. We have now found the morphological substrate of this enzyme by negative staining and by freeze-etching (Schmidt, Winkler and Plattner, 1981). About 2o particles of 1o nm diameter are present on the outer surface of chromaffin granules. They resemble closely the stalked particles observed previously in mitochondria.

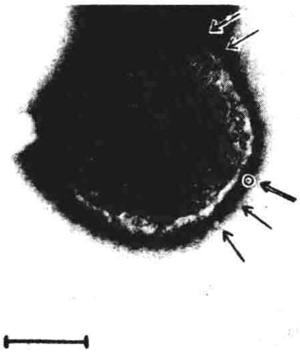

Fig. 1. Negative staining of chromaffin granules. Isolated chromaffin granules were subjected to negative staining (2 % ammonium heptamolybdate). The arrows indicate 1o nm particles on the surface of a chromaffin granule. The double arrow indicates a particle which has been encircled by ink for better demonstration. The bar represents 1oo nm.

Recent studies on the nucleotide transport (Weber and Winkler, 1981, and unpublished observations) indicate that the carrier has a relatively broad specificity. In addition to ATP other nucleotides, e.g. UTP and GTP, are transported with similar affinity. However, even phosphoenolpyruvate, PO_4^{3-} and SO_4^{2-} are transported. On the other hand, acetate, glutamic acid or pyruvate have no affinity for the carrier.

Future studies will probably soon lead to the isolation of the carriers for catecholamines and nucleotides. The exact molecular transport of these compounds can then be established.

Molecular Mechanism of Catecholamine Release

Although exocytosis as the mechanism of catecholamine secretion was already established fifteen years ago (see Smith and Winkler, 1972 for references) the molecular mechanism of this process still eludes us. The only established fact is the involvement of calcium, whereas the role of contractile proteins (see Trifaró, 1978), of granule membrane methylation (Gagnon and Heisler, 1979), of phosphorylation (Amy and Kirshner, 1981), of lysolecithin (see Smith and Winkler, 1972) and of synexin (Creutz and co-workers, 1979) are still a matter of speculation.

Japanese scientists (Oka and co-workers, 1965) first showed that incubation of chromaffin granules with ATP-Mg^{2+} led to the release of catecholamines. It was speculated that this release reaction was involved in catecholamine secretion in vivo (Poisner and Trifaró, 1967; see also Pazoles and Pollard, 1978). However, experimental proof is still lacking and evidence not supporting this suggestion has recently been presented (Englert and Perlman, 1981). It will therefore be one of the greatest challenges in the adrenergic field to elucidate the molecular mechanism of catecholamine secretion. This will not only be relevant for the adrenal medulla but also for other secretory systems where knowledge on this mechanism is still lacking.

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