Bacterial Toxins and Cell Membranes

Edited by

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Preface

The goal of this volume is to summarize recent knowledge about the interaction of toxins at the cell membrane level. It is also, in our opinion, very important to put together old and new information about biological effects of toxins to gain insight into their modes of action which are not clearly understood both from experiments on tissue culture cells and experiments in organ cultures or whole animals. Thus, it was not our intention that the contributors to this volume should cover work in detail which had been summarized in previous reviews, but to focus on certain aspects of phenomena at the cellular level or in the intoxicated animal which have been studied more recently. Authors were given a brief to formulate important questions that should be tackled concerning the mode of action of microbial toxins in the next ten years. Speculations by the authors were also encouraged and in this respect considerable latitude was permitted in the hope that this would provoke interest and experiment. The rapid increase in knowledge about cell membranes ought to promote such studies among toxinologists. However, it is hoped that this volume will make membranologists consider toxins as interesting potential probes in their studies and that the gap between the two groups will decrease rapidly.

Each chapter of this volume reflects the opinions of the authors. It has been our intention as editors not to influence the content of each chapter or the personal style of the authors, but to retain their intentions within a consistent format of subheadings and disposition for each chapter. This also means that some overlap cannot be avoided. In our view this has not been a great problem despite the fact that the subjects of different chapters inevitably overlap to some extent. Contact was maintained with each author during the writing of each chapter to prevent the most obvious repetition of data in different chapters.

Our apologies are extended to each contributor for any inconvenience caused during the preparation of individual contributions. Unfortunately, this volume was delayed nearly one year due to the fact that several chapters were not submitted on time for unforeseen reasons. In particular Drs Okamoto and Gill who delivered manuscripts in time for the first deadline for the whole volume must be thanked for their patience and perseverance.

Finally our thanks are due to Academic Press for their encouragement, patience and help throughout the preparation of this volume.

Warsaw and Uppsala
January 1978

J. JELJASZEWICZ and
T. WADSTRÖM

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1. Introductory overview

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The history of toxins is quite long, nearly one hundred years, compared to the rapid advance in membrane chemistry in the last two decades. The primary structures and functions of nucleic acids and proteins and their roles as functional and instructional molecules were discovered in the 1950s and 1960s. In the 1970s much interest has focused on membranes. Scientists from different areas of biology and medical sciences now study membranes and membrane-linked phenomena. Protein and lipid chemists, neurophysiologists and immunologists are just some groups with important questions connected with membranology. Many reviews and books in recent years have dealt with the structure and function of biological membranes (Chapman, 1968; Rothfield, 1971; Juliano, 1973; Zwaal *et al.*, 1973; Estrada-O and Gitler, 1974; Fleischer and Packer, 1974; Weissmann and Claiborne, 1975; Hatefi and Djavadi-Ohaniance, 1976; Maddy, 1976; Jamieson and Robinson, 1977; Rothman and Lenard, 1977). Current views on the mammalian cell membrane are largely based on studies concerned with erythrocyte plasma membranes. Subcellular membranes have been much less extensively studied.

Many important questions in membranology have not been answered yet (Rothman and Lenard, 1977). Our knowledge about the erythrocyte membrane concerning lipid and protein composition and membrane-associated phenomena such as active transport is quite advanced compared to what is known about the structure and function of the plasma membrane in other mammalian cells.

The rapid advances in membranology and the use of bacterial phospholipases and neuraminidases in studies on membrane structure and function and the use of cholera toxin as a probe for cellular functions regulated by cyclic AMP have greatly stimulated studies on microbial protein toxins and made this much more fashionable in the last decade, after the discovery of many toxins already at the beginning of this century. This volume reflects this steadily increasing interest in recent years.

The term "toxin" in this volume is used in its usual restrictive meaning employed by most bacteriologists to designate only products of relatively large molecular size and of protein or polypeptide nature which possess toxic properties in animal experiments or tissue cultures (Bonventre, 1970; Van Heyningen, 1970). The exception to this rule concerns bacterial endotoxin. Studies in this area in recent years have shown that the lipid part possesses most of the complex biological effects attributed to endotoxin. The extensive literature on biological effects of lipid A and endotoxin lies outside the scope of this volume and the particular contribution on endotoxin.

Bacterial protein toxins are probably mostly excreted by actively growing organisms or are sometimes products of cell lysis (Raynaud and Alouf, 1970). In contrast, endotoxin is located in the bacterial cell envelope and released into the surrounded medium. Bacterial endotoxins from different bacterial species have similar pharmacological properties, while bacterial protein toxins in different species have mostly quite unique biological and pharmacological properties.

Moreover, it should be mentioned that some protein toxins are composed of two or more separate protein entities which can act synergistically. This has been described for staphylococcal leucocidin (F and S components), succinic oxidase factor and γ-haemolysin, and also for a cytolytic toxin from *Streptococcus zymogenes* with haemolytic and bacteriocin properties (see Chapter 7). Recent studies on enterotoxins from different gram-negative species have shown that some of these crossreact immunologically and have similar or identical modes of action despite the fact that their bacterial sources are not closely related genetically. In some cases such similarities can depend on exchange of plasmid genes for toxin production between related species. However, it is still not known for several of these toxins if different serotypes exist (see Chapter 2).

Phospholipases of microbial and snake venom origin have proved to be very valuable analytical tools for study of membrane structure and function in recent years. The different specificities of these enzymes have made it possible to preferentially degrade phospholipids at the cell surface and have given us important evidence of a striking asymmetry in the major phospholipids in the erythrocyte membrane. A staphylococcal extracellular phospholipase with specificity for sphingomyelin has been especially useful in these studies. Other phospholipases from the gas gangrene bacterium (*Clostridium perfringens*) and from *Bacillus cereus* show other substrate specificities. The first two enzymes lyse erythrocytes of certain animal species with different phospholipid composition; the *C. perfringens* enzyme is highly toxic for animals and the main virulence factor in gas gangrene also in man (see Chapter 11).

Macfarlane and Knight (1941) postulated that the phospholipase, haemolytic

and dermonecrotic properties of crude *C. perfringens* toxins were due to one single protein molecule. However, it was not until the last decade that modern protein separation methods made it possible to prove that this hypothesis was right. It is now evident that this was the first discovery in microbial toxinology on the mode of action of a toxin at the molecular level. Yet, it still remains to be shown if this phospholipase and other microbial enzymes of this group with toxic properties possess additional properties which make them able to penetrate to their substrates in the membrane. However, it is known that another toxin which has no enzymatic activity, namely staphylococcal α-toxin, has surface-active properties and binds to receptors in membranes before inducing cell lysis. The binding of α-toxin to erythrocytes was found to correlate with their sensitivity to α-toxin-induced haemolysis, whereas artificial lipid vesicles (liposomes) made from lipid extracts of human and rabbit erythrocyte membranes were equally susceptible to lysis by α-toxin (see Chapter 4).

Other microbial enzymes which could affect membrane structure and function are different glycosidases including neuraminidase, certain endoglycosidases and different proteases. However, no toxic effects have been ascribed to any of them, probably except for protease with elastase activity from *Pseudomonas aeruginosa* and certain other gram-negative bacterial species. However, these toxic effects are probably caused by degradation of the intercellular matrix rather than due to a primary membrane effect.

The structural functional aspects of diphtheria toxin, tetanus toxin and cholera toxin have been studied in great detail in recent years. The intracellular events upon intoxication with diphtheria toxin have been elucidated, while the early stage of binding of the toxin to the cell plasma membrane is not known. On the contrary, studies by three different groups published in 1973 revealed that the GM_1 ganglioside was the cell receptor for cholera enterotoxin; however, the later stage of penetration of the A fragment of this enterotoxin and the intracellular events leading to increase in the cell content of cyclic AMP and secondary effects such as water and ion secretion of intestinal cells are still incompletely understood. The rapid increase in our knowledge of cholera toxin is covered in two chapters (see Chapters 9 and 10). The rapid progress in this field of microbial toxinology has encouraged many groups to study different microbial toxins at the cell level. Such studies have already made cholera toxin, besides phopholipases, an important probe in studies of the structure and function of mammalian cell membranes.

Cholesterol is an important constituent of mammalian membranes, but is not found in microbial plasma membranes except for certain *Mycoplasma* and *Acholeplasma* species. Although the phospholipid composition in different species of erythrocytes and other cells varies considerably, the cholesterol content is relatively constant and exists in approximately equimolar concentra-

tions with phospholipids. Some of the cholesterol is relatively easy to extract with organic solvents and it has been demonstrated that cholesterol can modify the fluidity of the membrane. A group of bacterial toxins called oxygen-labile (thiol-activated) haemolysins from a variety of species bind to cholesterol in mammalian membranes. Much evidence for this has come forward in recent years (see Chapter 5). Such studies have revealed that these toxins are cytolytic with a high specific activity, but also affect cellular functions of a variety of mammalian cells at doses sublytic for erythrocytes.

The literature on staphylococcal and streptococcal toxins is very extensive and has also been reviewed in recent years. However, studies on the mode of action of different toxins alone in well controlled experiments should take due account of the complexity of possible synergistic and antagonistic effects and tell us about their possible role in pathogenicity beyond the cell membrane level. Thus collection of more data on effects of different bacterial toxins in different cell model systems could help us to a better understanding of certain cell functions. It is to be hoped that during the next ten years new data about the mode of action of certain microbial toxins will make some of them, besides certain phospholipases and cholera toxin, important tools in studies of different cell phenomena similar to the use today of different lectins with different sugar specificities in immunobiology and for purification of cell receptors and cell surface glycoproteins involved in receptor functions for hormones and protein toxins.

It took nearly twenty years to make membranologists aware of the fact that an enzyme (α-toxin) produced by the gas gangrene bacillus, could be used in studies of membrane phenomena. A timely contribution bringing the potential of other bacterial cytolytic toxins to the attention of membranologists has been written by Bernheimer (1974). Although certain toxins have gained respectability in their usage, other potentially interesting probes are still unfortunately relegated to the occult. Moreover, it is only in the last few years that microbial phospholipases, neuraminidases and toxins, such as cholera toxin, of high purity have become available for use as probes in membrane studies. Thus, many old observations have to be repeated and reconfirmed with such probes of high purity which unfortunately are not yet commercially available. Membranologists have to be made aware of certain pitfalls and the erroneous conclusions that can arise from the use of impure reagents, e.g. *C. perfringens* phospholipase contaminated with the cholesterol binding cytolytic toxin (θ-toxin), neuraminidase and a few proteases all secreted from the cell of *C. perfringens* during growth. Even methods such as affinity chromatography have failed to separate such contaminants completely. Several criteria of purity have to be fulfilled before these reagents are used in membrane studies. Indeed, similar problems have been encountered in using other substances like lectins in membrane studies.

Advances in the area of the biochemical nature of the substrates or binding sites or receptors for bacterial toxins have been slow since the initial breakthrough with *C. perfringens* phospholipase C (Macfarlane and Knight, 1941). As yet only a handful of toxins have had their substrates or receptors defined and widely accepted, e.g. SH-activated cytolysins (cholesterol) and cholera toxin (GM_1 ganglioside). In other instances, toxins which have been extensively studied in the past decade using new purification techniques and which have been characterized with respect to physical/chemical properties and biological activity have elluded attempts to elucidate their membrane receptors, e.g. staphylococcal α- and δ-toxins. As will be clear from the various contributions to this volume, it is generally agreed that definition of membrane targets or receptors is essential for better understanding of the mode of action of toxins at the molecular level and to their potential usefulness as probes of membrane structure and function.

Although this book is devoted to bacterial toxins and membranes it is worth pointing out that plant and animal toxins could also prove to be good candidates as probes in the hands of membranologists. For example, phallolysin, a cytolytic toxin from the green bulbous mushroom *Amanita phalloides* binds to N-acetylglucosamine residues (Faulstich and Weckauf, 1975) and *Stoichactis helianthus* toxin, a cytolysin from the nematocysts of this coelenterate, appears to bind to sphingomyelin (Bernheimer and Avigad, 1976). Thus, although phospholipases and cholera toxin have provided new insight into the structure and function of membranes and the mechanisms of membrane-associated phenomena, biological reagents such as toxins which are neither degradative nor activate adenyl cyclase could clearly contribute to investigations within the broad scope of membrane biology. It is hoped that the following contributions will provide the necessary stimulus to this end.

The rapid advances in studies on enzymes and toxins from snake venoms and bacteria in recent years seem encouraging for the future. Studies on the primary structure of phospholipases and neurotoxins from snake venoms and phospholipase A_2 from pig pancreas (Avigad, 1976) and a deeper understanding of their toxic properties and their different biological effects should provide a stimulus for similar studies on enzymes and toxins of microbial origin. The regular symposia on animal, plant and microbial toxins (Ohsaka and Hayashi, 1976a, b) will hopefully encourage biologists in various research fields to study their modes of action at the molecular level

The development of antibiotics in the last three decades has slowed down basic research on microbial toxins and pathogenicity factors. However, interest in mechanisms of pathogenicity both at the molecular and/or cellular level and in the whole animal is now rapidly increasing again, a fact reflected by several reviews and symposia in the last few years (Smith and Pearce, 1972; Schlessin-