Advances in Planar Lipid Bilayers and Liposomes

Volume 3

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PREFACE

Volume 3 presents recent research on Bilayer Lipid Membranes (BLMs) based on a historic perspective of the lipid bilayer concept and its experimental realization. Many of the contributing authors were in close collaboration with the late Prof. H. Ti Tien, the founding editor of this series, on the use of supported BLMs for biosensors and molecular devices development.

In 1961 at the Symposium on the Plasma Membrane, when a group of researchers (Rudin, Mueller, Tien and Wescott) reported the reconstitution of a bimolecular lipid membrane *in vitro*, the report was met with skepticism. Those present included some of the foremost proponents of the lipid bilayer concept, such as Davson, Danielli, Stoeckenius, Adrian, Mauro, Finean and many others.

The research group began the report with a description of mundane soap bubbles, followed by 'black holes' in soap films, ... ending with an invisible 'black' lipid membrane, made from lipid extracts of cow's brains. The reconstituted structure (6–9 nm thick) was created just like a cell membrane separating two aqueous solutions. As one of the members of the amused audience remarked, "... the report sounded like ... cooking in the kitchen, rather than a scientific experiment!" That was in 1961, and the first report by the group was published a year later. In reaction to that report, Bangham, the originator of liposomes, in an article written in 1996 entitled 'Surrogate cells or Trojan horses': "... a preprint of a paper was lent to me by Richard Keynes, then Head of the Department of Physiology (Cambridge), and my boss. This paper was a bombshell ... . They (Rudin, Mueller, Tien and Wescott) described methods for preparing a membrane ... not too dissimilar to that of a node of Ranvier ... . The physiologists went mad over the model, referred to as a 'BLM,' an acronym for Bilayer or by some for Black Lipid Membrane. They were as irresistible to play with as soap bubbles"

Indeed, the group under the leadership of D.O. Rudin, then working in Philadelphia, PA, on the 9th floor, at Eastern Pennsylvania Psychiatric Institute (now defunct), was playing with soap bubbles with the 'equipment' purchased from the local toyshop! While nothing unusual for the researchers at work, it must have been a curious and mysterious sight for the occasional visitor who happened to pass through the laboratories there!

Today, after four decades of research and development, BLMs (also referred to nowadays as planar lipid bilayers), along with liposomes, have become established disciplines in certain areas of membrane biophysics and cell biology and in biotechnology. The lipid bilayer, existing in all cell membranes, is most unique in that it serves not merely as a physical barrier among cells, but functions as a two-dimensional matrix for all sorts of reactions. Also, the lipid bilayer, after suitable modification, acts as a conduit for ion transport, as a framework for antigen–antibody binding, as a bipolar electrode for redox reactions, and as a reactor for energy conversion (e.g. practical AIDS research, and 'microchips' study. In reactions involving light, BLMs have provided insights into the conversion of solar energy *via*, water photolysis, and to photobiology comprising apoptosis and

photodynamic therapy. Supported bilayer lipid membranes (s-BLMs) are being used in biosensor development. In addition, this volume reviews the studies of others in collaboration with our laboratory and also recent research of others on the use of BLMs as models of certain biomembranes.

With this background in mind, the present volume of the *Advances* series on planar lipid membranes and liposomes not only continues to include invited chapters on a broad range of topics, ranging from theoretical research to specific studies and experimental methods, but also refers to practical applications in many areas. The author of each chapter presents the results of his/her laboratory. We continue in our endeavor to focus in this series on newcomers in this interdisciplinary field, but we also wish to attract experienced scientists. We also try to show the importance of BLM applications for further development of this scientific research worldwide. The contributed chapters are separate entities to themselves, but they have one common feature. They are based on lipid bilayer concept of biomembranes, and have a significant impact to further development of the lipid bilayer research. We are grateful to all contributor(s) for their expert knowledge in BLM research area, for the shared information about their work and also for their patience in preparation of this volume after the unexpected death of the founding editor Prof. H. Ti Tien. Their willingness to write these chapters in his memory is much appreciated by the whole scientific community.

The first stage of the editorial work on this volume is still based on a joint effort of the late Prof. H.T. Tien and me. I would like to express my gratitude once more to everybody who contributed a chapter to this volume. I value the support and help of Dr. Kostas Marinakis, Publisher of Chemistry and Chemical Engineering Department in Elsevier and all his coworkers, especially Deirdre Clark, in different stages of the preparation of this series. We will try our best to keep this *Advances* series alive and to pay our tribute to the scientific works of Prof. H. Ti Tien.

Angelica Leitmannova Liu
(Editor)

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CONTRIBUTORS

CHAPTER 1

Supported Lipid Bilayers for the Detection of Hormone-Receptor Interactions

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PROLOGUE

Hormone–receptor interactions play a key role in controlling balanced homeostasis. A hormone is a naturally occurring substance secreted by specialized cells that affect the metabolism or behavior of other cells possessing functional receptors for the hormone. The interaction between a hormone and its complementary receptor stimulates a physiological response in the target cell.

Cell membranes are composed of lipids, proteins, carbohydrates, and cholesterol in the form of a phospholipid double (bilayer) containing phospholipid molecules with a long hydrophobic tail and a hydrophilic head group embedded in the membrane proteins. As the structure of the lipid bilayer prevents the free passage of hormones into and out of the cell, specific receptors are required to mediate the interaction between the extracellular membrane phase and the intracellular volume. Receptors belong to a special class of transmembrane proteins that bind signaling molecules. Following binding, the receptor acts as a signal transducer that converts an extracellular ligand-binding event into an intracellular signal, thereby altering the activity of the target cell.

The universally studied model system for the cell membrane is a synthetic phospholipid bilayer that serves as a physical barrier between liquid compartments. Furthermore, the bilayer can function as two-dimensional (2D) matrix for biological reactions. Biological activity is accomplished by suitable modification of the bilayer, such as embedding biologically active molecules like receptors, ion-channels, antigen, antibody, and signal transduction proteins.

This chapter describes the contribution of a biologically modified supported bilayer in the field of endocrinology and its importance to pharmacology, epidemiology, virology, medicine, and environmental sciences. The binding of hormone to its receptor activates a cascade of reactions within the cell that affect physiological and biological function.

1. HORMONES

Cells communicate through chemical signals. A hormone is defined as a complex signal molecule produced in a specialized cell that elicits a physiologic response in another cell bearing a receptor for the hormone. Thus, hormone systems can be described as a broadcast of long-lasting chemical messengers. Hormones can be hydrophilic, in which case the receptors are on the cell surface, or lipophilic, in which the receptor can be intracellular. Although certain hormones can circulate dissolved in the blood, most are bound to plasma proteins.

Hormones produced by the endocrine glands are secreted by cells directly into the general circulation, exerting a physiologic effect on target cells located at distant sites. The endocrine chemical communication system comprises the pituitary gland, pineal body, thyroid and parathyroid glands, adrenals, pancreas,

Table 1. Human hormones produced by the endocrine system

Hormone	Principal source
Thyroid-stimulating hormone (TSH)	Pituitary gland
Follicle-stimulating hormone (FSH)	Pituitary gland
Luteinizing hormone (LH)	Pituitary gland
Growth hormone (GH)	Pituitary gland
Adrenocorticotropic hormone (ACTH)	Pituitary gland
Gonadotropin-releasing hormone (GnRH)	Pituitary gland
Dopamine	Pituitary gland
Melatonin	Pineal body
Throxin (T4)	Thyroid Gland
Parathyroid hormone (PTH)	Parathyroid glands
Glucocorticoids (e.g., cortisol)	Adrenal cortex
Mineralocorticoids (e.g., aldosterone)	Adrenal cortex
Androgens (e.g., testosterone)	Adrenal cortex
Adrenaline (epinephrine)	Adrenal medulla
Noradrenaline (norepinephrine)	Adrenal medulla
Estrogens (e.g., estradiol)	Ovarian follicle
Progesterone	Corpus luteum and placenta
Human chorionic gonadotropin (HCG)	Trophoblast and placenta
Androgens (e.g., testosterone)	Testes
Insulin	Pancreas (Islets of Langerhans)
Insulin-like growth factor (IGF-1)	Liver

and gonads (testes and ovaries). A partial list of human hormones produced by the endocrine glands is presented in Table 1.

1.1. Classification of hormones

Hormones are derived from amino acids, cholesterol, or phospholipids (Fig. 1). Chemically, hormones are classified as (1) proteins or peptides, (2) modified amino acids, or (3) steroids.

1.1.1. Proteins and peptides

The size of protein or peptide hormones ranges from as few as 3 amino acids to over 200 residues. One group of peptide hormones, represented by insulin, consists of two subunits attached by disulfide bonds between two cysteine molecules. The more complicated structures are the glycoprotein hormones. For example, the anterior pituitary gland hormone comprises two protein subunits attached by complex sugar moieties [1].

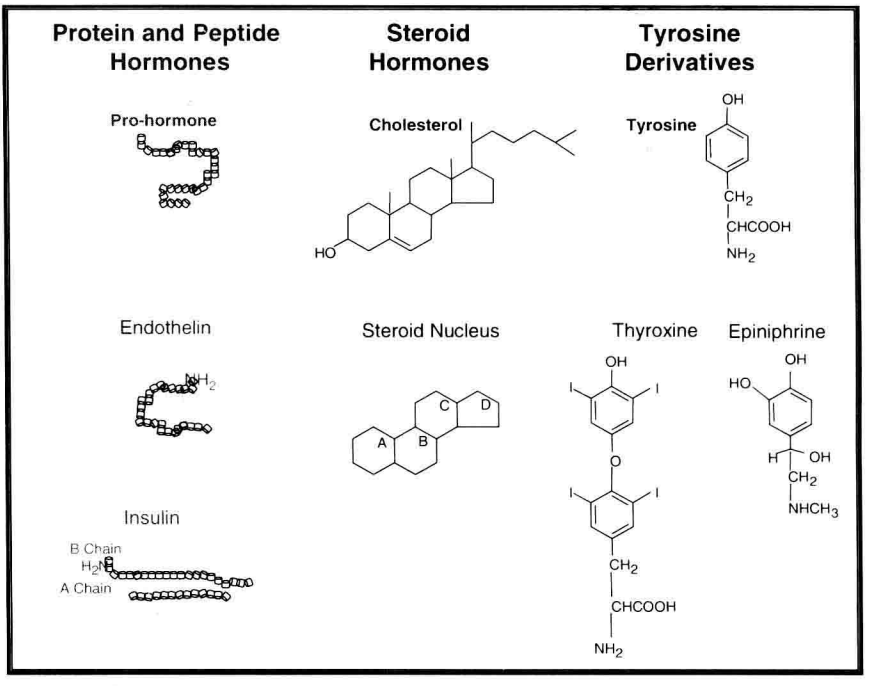

Fig. 1. Schematic demonstration of hormone classification.

1.1.2. Modified amino acids

Amine hormones are derived from amino acids – for example, the catecholamines (epinephrine (EP) (adrenalin) and norepinephrine), thyroxine from tyrosine, histamine from histidine, and serotonin from tryptophan. The activity of norepinephrine as both neurotransmitter and hormone is just one example of the complexity of endocrine balance and control. The catecholamine hormones are secreted by the adrenal medulla and are rapidly broken down once released into the circulation. Tryptophan is the precursor of serotonin (5-hydroxytryptamine) and melatonin synthesis. Thyroid hormones are formed by the conjugation of two tyrosine molecules.

1.1.3. Steroids

Steroid hormones are derived from cholesterol except for retinoic acid, which is derived from vitamin A. All adrenal and gonadal steroids (sex hormones), including vitamin D, have the same basic ring skeleton structure (Fig. 2). The specificity of steroid hormones derives from side chains, residues, and spatial orientation. Steroid hormones are transported bound to plasma proteins and typically react with receptor sites inside a cell. Thyroid hormones resemble steroid hormones in their binding to serum proteins and mechanism of action.

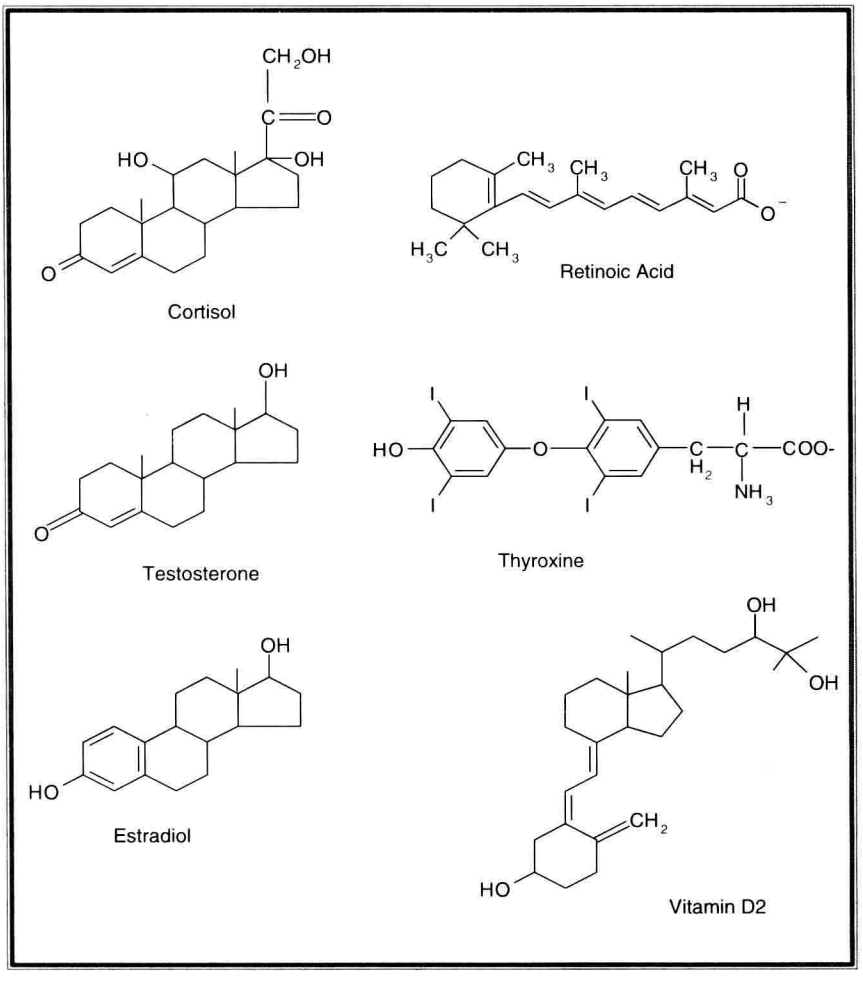

Fig. 2. Schematic ring skeleton structure of steroid hormones.

1.1.4. Phospholipids

Another group that should be mentioned includes hormones derived from lipids and phospholipids. This group includes the major classes of eicosanoids, including prostaglandins, prostacyclins, thromboxanes, and leukotrienes (Fig. 3).

1.2. Mechanism of action

A hormone elicits a response in its target cells following recognition by a cell-surface receptor protein specific for that particular hormone. This unique receptor molecule is the only receiver that can accept the message, recognize the specific hormone, and mediate the biological pathway to activate the intracellular response to the signal. The binding of hormones to their complementary receptors

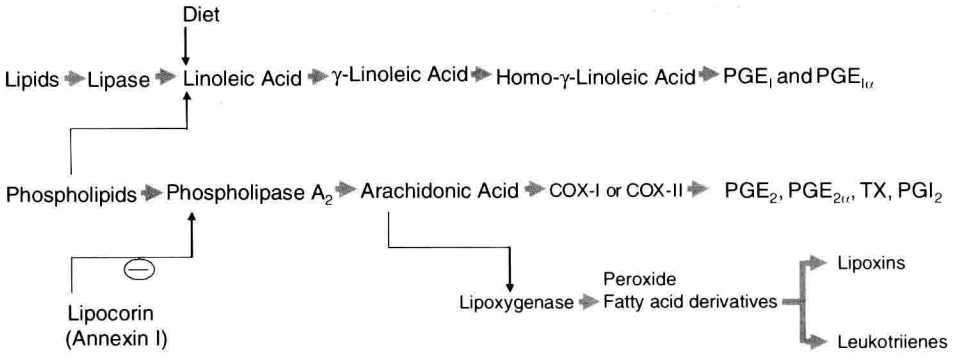

Fig. 3. Schematic description of hormones derived from lipids and phospholipids synthesis pathway. The described pathway products, prostaglandins (PGE), prostacyclins (PGI), thromboxanes (TX), and leukotrienes release to the cytoplasm direct membrane action for cellular response.

can either activate or inhibit various cellular functions. The diversity of receptor structures and functions is exceptionally broad.

1.2.1. Regulation

The absolute hormone concentration in plasma is of great importance in health balance. Any deviation from the physiological hormone concentrations is crucial and can result in acute disease. The parameters for achieving the precise local hormone concentration are hormone reproduction *versus* hormone degradation, hormone delivery rate, and the equilibrium between bound and free hormone.

The secretion of hormones is regulated by feedback loops that control the hormone concentration in the blood. Figure 4 schematically describes a simple feedback loop.

One example of the feedback mechanism in the human body is male sperm production in which the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the brain is followed by the production of testosterone, the main androgen (male sex hormone) produced by the testes. Sperm production, as well as several secondary male sex characteristics (deepening voice, beard growth, appearance of body hair), is derived from the amount of testosterone in the blood. When the testosterone concentration achieves the optimal concentration, sperm production is stimulated, whereas the production of the brain hormones (FSH, LH) is inhibited when testosterone is increased. This feedback loop is summarized in Fig. 5.

2. RECEPTORS

Cell-surface receptors are integral membrane proteins that interact with the hormone and stimulate direct or indirect response of the cell organelles, including the

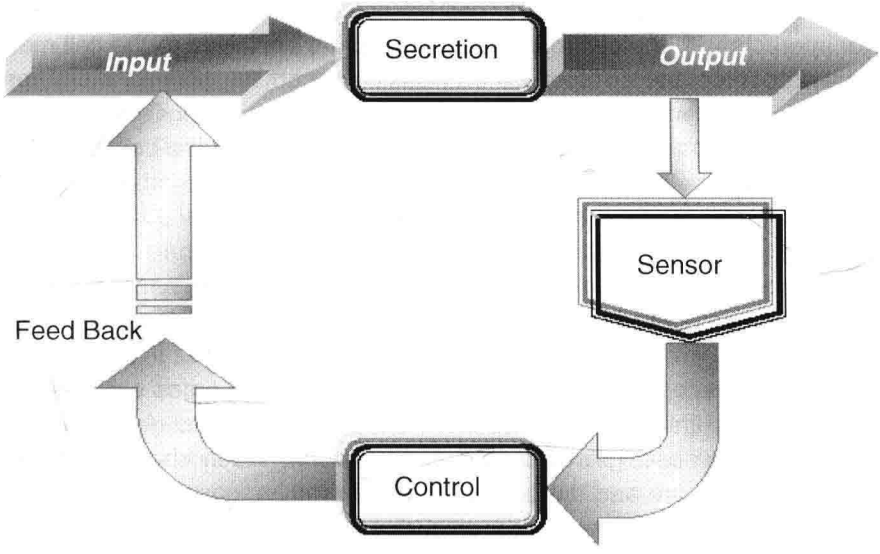

Fig. 4. Scheme of a simple feedback loop of a biological secretion system. The output of the secretion process is detected by a biological 'sensor'. The sensing mechanism is delivering the signal to a control system. The control system sends a signal to the input process, actually it is the feedback for the input.

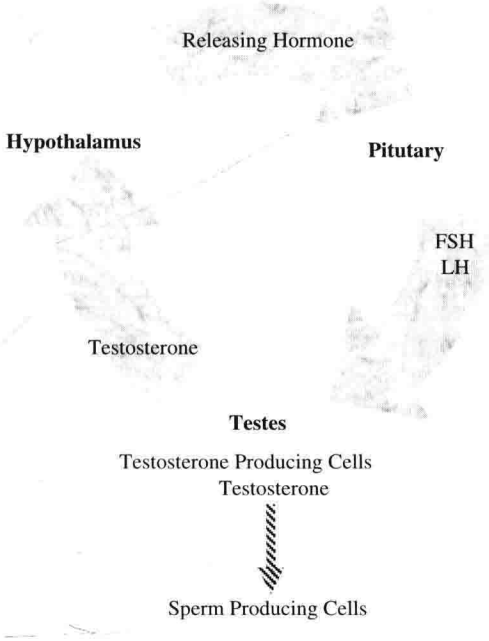

Fig. 5. Example of feedback loop balanced male sperm production.