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Peptide Hormones, Biomembranes, and Cell Growth

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PREFACE

The field of study of receptors today is one of the most innovative in biology and pathology.

This book provides a multidisciplinary approach to the problem: the biochemical characterization of binding, the mode of action of receptors, their relationship to ion transport as well as the relevance of membrane fluidity in receptor activity are discussed.

It is hoped that this volume will stimulate further collaboration among scientists in both basic and applied disciplines.

The thanks of all the participants are particularly due to Roberto Verna, who, with his efficiency and enthusiasm, has organized such an outstanding scientific meeting.

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CONTENTS

Introduction. 1
C.L. Bolis

INTERACTION OF PEPTIDE HORMONES WITH PLASMA MEMBRANES:

MODULATION OF MEMBRANE FUNCTIONS

What Binding Experiments Can and Cannot Tell Us About the Interaction Between Hormones and Membranes. 5
A.P. Minton

The Binding of Multivalent Effector to Membranes and Its Kinetic Regulation. 11
R. Strom

The Properties of Coated Vesicles and Their Role in Receptor-Mediated Endocytosis 23
H. Edelhoch and P.K. Nandi

Interaction of Insulin with Plasma Membrane of Target Cells: The Role of Membrane Fluidity 39
P. Luly

Evaluation of Target Cell Responsiveness to Peptide Hormones: A Possible Role for the Na/K ATPase 51
R. Verna, P. Braquet, J. Diez, and R. Garay

Regulation of Na^+ and K^+ Transport in Macrophages 63
P. Braquet, J. Diez, F.V. DeFeudis, R. Verna, and R. Garay

Chronobiology of the Na/K ATPase Activity in Human Erythrocyte Membranes 79
R. Verna, P. Braquet, P. Cugini, and R. Garay

The Involvement of cAMP in the Hormonal Regulation of Ion Transport. 89
R. Garay, J. Diez, R. Verna, C. Nazaret, and P. Braquet

Effects of Glucocorticoids on Hormonal Activation of Adenylate Cyclase. 97
G. Tolone, L. Bonasera, and P. Lo Presti

The Round Trip of Thyroglobulin Within the Thyroid Cell: Cellular Receptor(s) and Molecular Signals for the Recognition. 103
S. Formisano, L.D. Kohn, and E. Consiglio

Gonadotropin Regulation of Sertoli Cell Response 115
M. Conti, M.V. Toscano, R. Geremia, P. Rossi, L. Monaco, and M. Stefanini

New Class of Opioid Peptides: Dermorphins. 127
P. Melchiorri, G. Improta, L. Negri, and M. Broccardo

MEMBRANE-RELATED EFFECTS ON CELL METABOLISM AND GROWTH

Genetic Analysis of Cyclic AMP Response in Cultured Cells. 143
M.M. Gottesman, G. Vlahakis, and C. Roth

Growth Regulation in S49 Mouse Lymphoma: Involvement of Cyclic AMP and Substrate Adhesiveness. 155
J. Hochman and E. Levi

TSH Receptor Mediated Growth of Thyroid Cells: Mechanism and Relation to Autoimmune Thyroid Disease 169
S.M. Aloj, C. Marcocci, M. De Luca, S. Shifrin, E. Grollman, W. Valente, and L.D. Kohn

The Mitogenic Activity of Thyrotropin: The Role of cAMP. . . 197
D. Tramontano, R. Picone, B.M. Veneziani, G. Villone, and F.S. Ambesi-Impiombato

Relevance of Cellular Polyamines in the Response of Heart Cells to Cyclic Nucleotide-Mediated Effectors. 207
C. Clo, B. Tantini, C. Pignatti, S. Marmiroli, and C.M. Caldarera

Ionic Signal Transduction by Growth Factors. 215
W.H. Moolenaar, L. Joosen, and S.W. de Laat

Effect of a Conditioned Medium on the Growth Rate and Nutrient Transport in SV3T3 Cells. 233
L. Silvotti, P.G. Petronini, G. Piedimonte, and A.F. Borghetti

Osmoregulation of Growth Rate and Nutrient Transport in Cultured Fibroblasts. 239
P.G. Petronini, M. Tramacere, and A.F. Borghetti

Regulation of the Urokinase mRNA Synthesis by Tumor Promoters and Epidermal Growth Factor. 245
M.P. Stoppelli, P. Verde, P. Galeffi, E. Kajtaniak Locatelli, and F. Blasi

Control of Transmitter Release by Catecholamines and Prostaglandins 253
A. Capuzzo, C. Biondi, P.G. Borasio, E. Fabbri, E. Ferretti, and A. Trevisani

Ganglioside Involvement in Trophic Interactions 271
D. Benvegnù, D. Presti, L. Facci, R. Dal Toso, A. Leon, and G. Toffano

Concluding Remarks 283
G. Salvatore

Contributors 287

Index. 291

INTRODUCTION

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The importance of biomembranes as the recognition site for many hormones, neurotransmitters, toxins and drugs is now well documented. In addition, cell cycle steady states in actively growing cells seem to play an important role in the control of receptor density (Holley et al., 1977). For this reason it seems particularly important today to consider cellular activity as an essential part of the cooperativity of membranes in signal recognition and transduction (Bolis and Luly, 1978). For example, cell receptor numbers can be observed to change during cell cycle, cellular development and differentiation.

During the 1950's various experimental approaches were developed to study hormonal binding to target cells; later, radiolabelled hormones with high specific activities made available information on the binding characteristics and the specificity of hormones for specific receptor sites (Cuatrecasas and Hollenberg 1976). These studies have been conducted with intact cell preparations, purified or crude hormone preparations, and solubilized receptors (Kono, 1969; Roth, 1973). However, the experimental approaches all lack the objectivity of a study which takes into account simultaneously overall cellular activity as occurs *in vivo*. This is true even if we know that the timeless sequence of the binding induces a relevant response through physiological changes in the cell, involving plasma membrane linked phenomena, like adenylate cyclase activation and/or permeability changes (Haynes et al. 1960; Jarett and Smith, 1974).

Considerable progress in the understanding of the membrane structural organization in time and space has been achieved (Singer, 1976; Singer and Nicholson, 1972). The evidence that certain membrane proteins are free to diffuse in the plane of the plasma membrane allows one to consider the possibility that hormone receptors may also be mobile entities that interact with other molecules in the membrane's plane. This concept of mobile receptors developed by Jacobs and Cuatrecasas, 1976; De Haen, 1976; Boeynaems and Dumont, 1977, is based on observations that receptor-specific agonists (AcTH, prostaglandins, glucagon, catecholamines, etc.) independently can stimulate adenylate cyclase in the adipocyte; this offers a new approach to the multireceptors concept.

Receptors at all surface levels may be affected by several conditions secondary to intracellular events (cell cycle, cell differentiation and rate of synthesis and turnover) or by a variety of external stimuli determined by hormones and other agents (homospecific or heterospecific) or chemical toxins or virus (Hollenberg, 1979).

In this meeting, very interesting aspects of the role of membrane fluidity which relates to the mobility of molecules in the plane of the membranes, were discussed, as well as the kinetics regulating receptor binding events, and other molecular events underlying the hormonal effects.

Even more important is the pathology of hormone/receptor interaction, mainly due to both hormone and receptor concentration changes, alteration in affinities, as well as anti-receptor antibodies pathology (Roth and Taylor, 1982).

REFERENCES

Boeynaems, J. M. and Dumont, J. E., 1977, The Two-Step Model of Ligand-receptor Interaction. Mol. Cell Endocrinol., 7:33

Bolis, L., and Luly, P., 1978, Membrane Receptors, in: "First Symposium on Organ Specific Autoimmunity", P. Miescher, ed., Schwabe, Basle, p. 127

Cuatrecasas, P. and Hollenberg, M. D., 1976, Membrane Receptors and Hormone Action. Advan. Protein Chem., 30:251

Dehaen, C., 1976, The Non-stoichiometric Floating Receptor Model for Hormone-sensitive Adenylate Cyclase. J. Theor. Biol., 58:383

Haynes, R. C. et al., 1960, The Role of Cyclic Adenylic Acid in Hormone Action. Recent Progr. Hormone Res., 16:121

Hollenberg, M. D., 1979, Hormone Receptor Interactions at the Cell Membrane. Pharmacological Reviews, Vol. 30, No. 4

Holley, R. W. et al., 1977, Density-dependent Regulation of Growth of BSC-1 Cell Culture: Control of Growth by Serum Factors. Proc. Nat. Acad. Sci., 74:5046

Jacobs, S. and Cuatrecasas, P., 1976, The Mobile Receptor Hypothesis and "Cooperativity" of Hormone Binding Application to Insulin. Biochem. Biophys. Acta, 433:482

Jarett, L. and Smith, R. M., 1974, The Stimulation of Adipocyte Plasma Membrane Magnesium Ion-Stimulated Adenosine Triphosphatase by Insulin and Concanavalin A., J. Biol. Chem., 249:5195

Kono, T., 1969, Destruction and Restoration in the Insulin Effector System in Isolated Fat Cells. J. Biol. Chem., 244:5777

Roth, J., 1973, Peptide Hormone Binding to Receptors: A Review of Direct Studies In Vitro. Metabolism, 22:1059

Roth, J. and Taylor, S. I., 1982, Receptors for Peptide Hormones: Alterations in Diseases of Humans. Ann. Rev. Physiol., 44:639

Singer, S. J., 1976, The Fluid Mosaic Model of Membrane Structure Some Applications to Ligand-receptor and Cell-cell Interactions, in: "Surface Membrane Receptors", R. A. Bradshaw, W. A. Frazier, R. C. Merrell, D. I. Gottlieb and Hogue-Angeletti, R. A., eds., Plenum Press, New York

Singer, S. J. and Nicholson, G. L., 1972, The Fluid Mosaic Model of the Structure of Cell Membranes. Science, 175:720

WHAT BINDING EXPERIMENTS CAN AND CANNOT TELL US ABOUT THE INTERACTION BETWEEN HORMONES AND MEMBRANES

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INTRODUCTION

Until less than twenty years ago, information about the interaction between a hormone and its target tissue had to be indirectly inferred from the dependence of the magnitude of elicited response upon hormone dose or concentration. Following development of methods for the preparation of radiolabeled hormones of high specific activity (reviewed in Cuatrecasas and Hollenberg, 1976) it became possible to measure directly the binding of as little as a few picomoles of hormone to a target preparation. By correlating the extent of binding with the dose and with the extent of elicited response it became possible to identify, and in some cases, isolate, particular sets of binding sites termed receptors, the occupation of which by hormone is associated with (and assumed to be) the initiating event in the elicitation of response by hormone.

The ability of receptors to specifically bind a particular ligand has been exploited, on one hand, to facilitate the chemical isolation and characterization of the receptor molecule, and on the other hand, to explore the molecular mechanism by which binding of hormone to receptor leads to the observed cellular response. The purpose of this communication is to briefly review factors which can affect the binding of hormones to their receptors and to emphasize that binding data alone do not permit one to identify which of the possible contributory factors are actually operative in the system under investigation.

For the purpose of this work we shall use the term "binding" to refer exclusively to a specific, saturable interaction between hormone and one or more sets of binding sites. Experimental data

are assumed to have been corrected properly for the presence of weak, unsaturable adsorption of hormone to target tissue (commonly referred to as "nonspecific binding").

CLASSIFICATION OF BINDING ISOTHERMS

The functional dependence of binding upon ligand concentration at a single temperature is referred to as a binding isotherm. We shall classify isotherms on the basis of the appearance of the plotted data (Minton, 1982a).

The reference isotherm is that relation generated by the Langmuir equation

$$H_b/H_b^{max} = K\,[H] \,/\, (1 + K\,[H]) \qquad (1)$$

where H_b is the amount or concentration of bound hormone, H_b^{max} is the amount of bound hormone at saturation, [H] is the concentration of free (unbound) hormone, and K is an equilibrium constant for association of univalent hormone with a single class of independent, equivalent binding sites. The titration plot of the reference isotherm (Figure 1a, solid curve) is sigmoid, symmetrical about the half-saturation point, with two log units in free hormone concentration separating the 9 per cent and 91 per cent levels of site saturation. The Scatchard plot of the reference isotherm (Figure 1b, solid curve) is a straight line.

Two other general types of isotherm frequently observed are schematically depicted in Figures 1a and 1b. Curves resembling those drawn with short dashes in the figures will be referred to as apparent cooperative isotherms. Less than two log units in free hormone concentration separate the 9 per cent and 91 per cent levels of site saturation in a titration plot, and the Scatchard plot is convex upward. Curves resembling those drawn with alternate dots and dashes in the figures will be referred to as apparent multiple site class isotherms. More than two log units in free hormone concentration separate the 9 per cent and 91 per cent levels of site saturation in a titration plot, and the Scatchard plot is concave upward.

FACTORS WHICH MAY INFLUENCE THE BINDING OF HORMONE TO MEMBRANE RECEPTORS

While the interpretation of a reference-type isotherm in terms of a single homogenous (or quasi-homogeneous) class of independent binding sites is straightforward, both the apparent multiple site and apparent cooperative types of isotherms may result from a variety of underlying causes. These are listed for reference in Table I and briefly discussed below.

1. If binding sites for hormone are partitioned into two or more discrete, independent classes of significantly different affinity for hormone, an apparent multiple-site class isotherm will result (Scatchard, 1949).

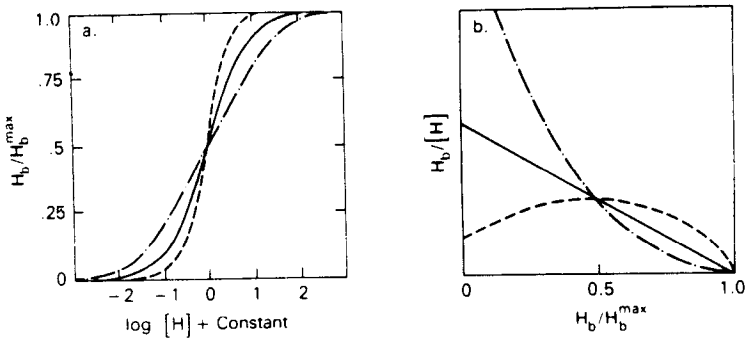

Fig. 1. Titration plot (a) and Scatchard plot (b) of three phenomenological types of binding isotherms. Reference isotherm, ——— ; apparent cooperative isotherm ------ ; apparent multiple site class isotherm, -·-·-·-· (Adapted from Minton, 1982a).

Table 1. Possible interpretations of isotherm shape.

Reference	Single homogeneous class of independent binding sites "Quasi-homogeneous" distribution of site affinity (Minton, 1979)
Apparent multiple site class	Multiple discrete classes of independent binding sites (Scatchard, 1949) Continuous distribution of site affinity (Karush and Sonnenberg, 1949) Negatively cooperative interaction between binding sites or hormone molecules (Scatchard, 1949) Nonequilibrium effects (Minton, 1982b) Nonstoichiometric receptor-effector interaction (Jacobs and Cuatrecasas, 1976)
Apparent cooperative	Positively cooperative interaction between binding sites or hormone molecules (Scatchard, 1949) Nonequilibrium effects (Minton, 1982b)

2. If binding sites for hormone consist of a group of closely related but nonidentical molecules (such as a glycoprotein of variable carbohydrate composition), or if the local environment of a receptor molecule varies from point to point over the membrane surface, a distribution of affinity for hormone may result, together with a correspondingly broadened (multiple site type) isotherm (Karush and Sonnenberg, 1949).

3. The affinity of a particular receptor molecule for hormone may depend upon the occupancy state of neighboring receptor molecules. If the occupancy of a receptor molecule by hormone decreases the affinity of a neighboring receptor molecule for hormone, a negatively cooperative interaction is said to exist between receptor molecules, and the binding isotherm will be broadened. If the occupancy of a receptor molecule by hormone increases the affinity of a neighboring receptor molecule for hormone, a positively cooperative interaction is said to exist between receptor molecules, and the binding isotherm will be steepened (Scatchard, 1949).

4. Cooperative interactions may exist between hormone molecules as well as between receptor molecules. One type of negatively cooperative interaction could result from the clustering of receptors into patches of high surface density. If the hormone molecule were large enough, simple steric repulsion between hormone molecules might prevent complete saturation of all binding sites in a patch, and the resulting binding isotherm would be greatly broadened.

5. If binding of hormone alters the rate of a reaction taking place continuously during the binding assay, a steady state far from equilibrium may be achieved in which the hormone binding isotherm may be either steepened or broadened (Minton, 1982b).

6. If binding of hormone to receptor alters the interaction between receptor and another macromolecular component of the target membrane (called effector), and if effector is present in a stoichiometric ratio to receptor less than unity, then an apparent two site class isotherm may result, reflecting the difference in affinity for hormone between receptor which interacts with effector, and receptor which is in excess of effector and cannot interact with it (Jacobs and Cuatrecasas, 1976).

It should be evident at this point that one cannot discriminate between the various alternatives offered above on the basis of binding studies carried out in tissue or membrane homogenates alone. However, a combination of binding measurements carried out on "intact" membranes and upon purified or partially purified membrane components may permit the range of choice to be substantially narrowed. An example of such a combined study may be found in Maturo and Hollenberg (1978).

INTERPRETING THE COMPETITIVE BINDING ASSAY: A CAUTIONARY NOTE

The competitive binding assay provides a convenient method for comparing the binding properties of a native hormone and chemically related substances, such as hormone analogs, which bind to the same receptors. Under the proper conditions the competitive assay may also provide quantitative binding isotherms for hormones which cannot be themselves radiolabeled without substantial loss of native binding or functional properties.

A conventional competition assay is carried out as follows (Campfield, 1983). A tracer substance (denoted here by B) is selected on the basis of two criteria: it may be radiolabeled to high specific activity, and it is displaced from its specific binding sites by a sufficiently high concentration of the unlabeled competitor (denoted by A). A series of assay samples are prepared containing fixed amounts of target tissue or membrane and tracer and varying amounts of the test substance A. The amount of bound B (B_b) is measured as a function of the concentration of unlabeled competitor ([A]). It is usually assumed that y_A, the fractional saturation of binding sites by A, is given by

$$y_A = A_b/A_b^{max} = 1 - B_b/B_b^o \qquad (2)$$

where B_b^o is the amount of B bound in the absence of A.

We would like to emphasize here two points which seem to have been generally neglected when applying eqn (2) to the analysis of competition data.

The first point is that the binding of A to membrane may not be adequately represented by the single species AR (where R denotes receptor). In the most general case, A may conceivably form a variety of complexes with R which may involve additional membrane components S, T, ... as well. Each of these complexes may be denoted by the general form $A_mR_nS_pT_q$ ..., where $m,n \geq 1$ and $p,q, \ldots \geq 0$. It is possible to show rigorously (A. Minton, unpublished calculations) that eqn (2) can only be valid if $m = n$ for each and every species of complex involving A and R. In particular, eqn (2) cannot be validly used if complexes of the form AR_2 and/or A_2R constitute a significant fraction of bound A.

The second point generally overlooked in the application of eqn (2) is that even when it is valid, the binding isotherm derived for A is only equal to that obtained via a direct binding assay when $B_b^o << B_b^{max}$, that is, when the fractional saturation of binding sites by B is negligible. If the fractional saturation of binding sites by B is non-negligible, then A_b (and y_A) are

functions of both [A] and [B], and may have values which are significantly less than those obtained for the same value of [A] in the absence of B.

REFERENCES

Campfield, L. A., 1983, Mathematical analysis of competitive protein binding assays, in: "Principles of Competitive Protein Binding assays", 2nd ed., W. D. Odell and P. Franchimont, eds., Wiley and Sons, New York.

Cuatrecasas, P. and Hollenberg, M. D., 1976, Membrane receptors in hormone action, Advances in Protein Chem. 30:252.

Jacobs, S. and Cuatrecasas, P., 1976, The mobile receptor hypothesis and 'cooperativity' of hormone binding, Biochim. Biophys. Acta 433:482.

Karush, F. and Sonnenberg, M., 1949, The interaction of homologous alkyl sulfates with bovine serum albumin, J. Am. Chem. Soc. 71:1369.

Maturo, J. M. III and Hollenberg, M. D., 1978, Insulin receptor: interaction with nonreceptor glycoprotein from rat liver membranes. Proc. Natl. Acad. Sci. U.S.A. 75:3070.

Minton, A. P., 1979, Apparent homogeneity in some heterogeneous systems, Biochim. Biophys. Acta 558:179.

Minton, A. P., 1982, Steady state relations between hormone binding and elicited response: quantitative mechanistic models, in: "Hormone Receptors", L. D. Kohn, ed., Wiley and Sons, New York.

Minton, A. P., 1982b, Elicitation of cellular response by bioactive ligands: nonequilibrium effects, Biochem. Biophys. Res. Commun. 107:1206.

Scatchard, G., 1949, The attraction of proteins for small molecules and ions, Ann. N.Y. Acad. Sci. 51:660.