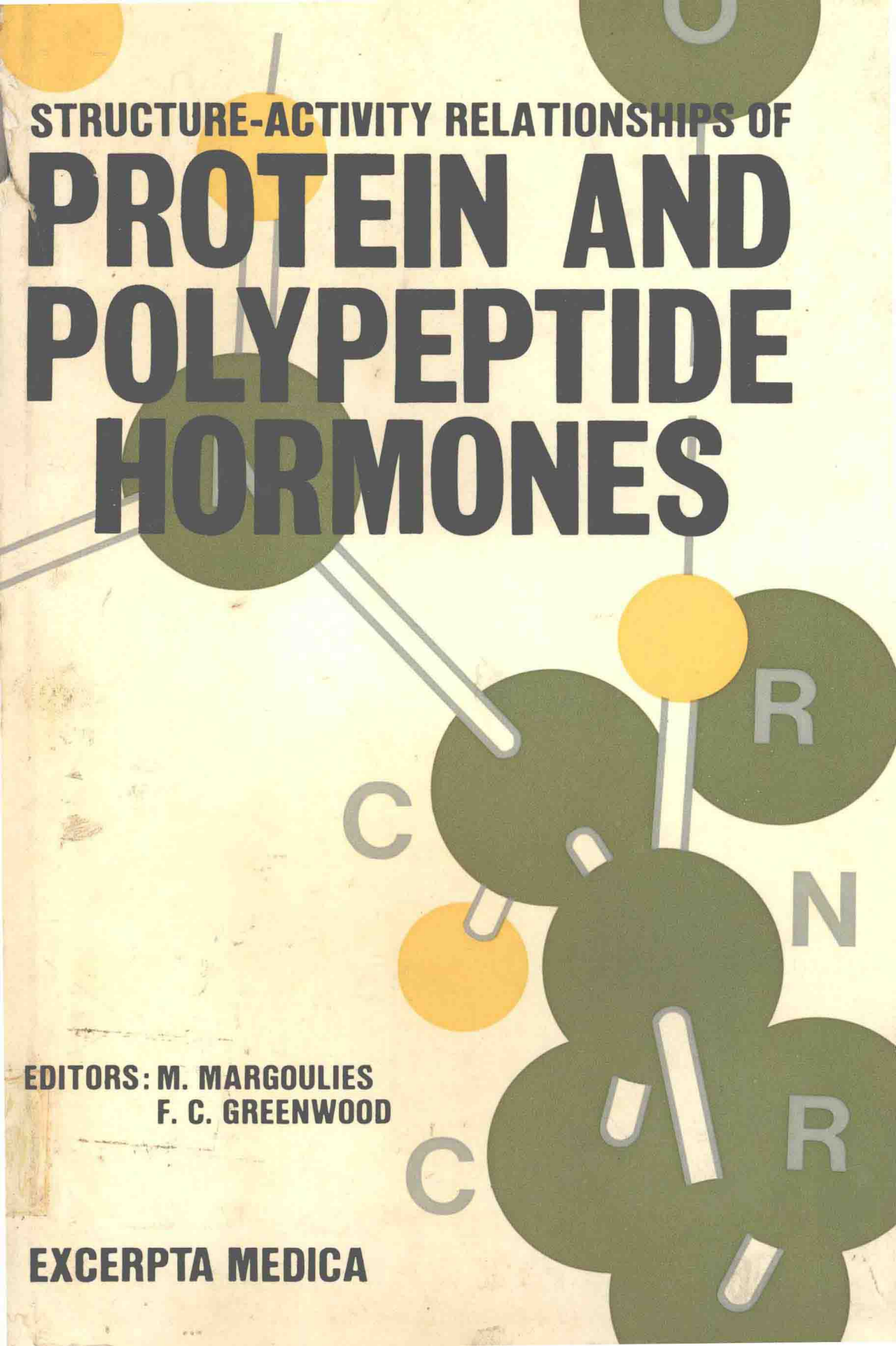
STRUCTURE-ACTIVITY RELATIONSHIPS OF PROTEIN AND POLYPEPTIDE HORMONES



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F. C. GREENWOOD

EXCERPTA MEDICA

STRUCTURE-ACTIVITY RELATIONSHIPS OF PROTEIN AND POLYPEPTIDE HORMONES

PROCEEDINGS OF THE SECOND INTERNATIONAL SYMPOSIUM
LIÈGE, SEPTEMBER 28-OCTOBER 1, 1971

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1972

EXCERPTA MEDICA

INTERNATIONAL CONGRESS SERIES NO. 241
ISBN 90 219 0172 2

EXCERPTA MEDICA OFFICES

Amsterdam Jan van Galenstraat 335
London Chandos House, 2 Queen Anne Street
Princeton Nassau Building, 228 Alexander Street

Printed in The Netherlands by A. W. Sijthoff, Leyden

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FOREWORD

The First International Symposium on Protein and Polypeptide Hormones held in Liège in May 1968 allowed us to try out a new kind of Symposium principally in the form of discussions.

With this aim in view, the main papers were published and put at the disposition of all the participants about a month before the meeting and were not read during the sessions. On the contrary, these were devoted in their entirety to the presentation of new facts and to their discussion.

This formula proved to be particularly useful and effective, and its significance was borne out in the volume of discussions published after the Symposium.

It was therefore decided to organize the Second International Symposium on Protein and Polypeptide Hormones by adhering to the same schema. Whereas the 1968 Symposium covered a considerable number of aspects of experimental hormonology (radioimmunoassay, structure-activity relationships, lipolysis, lipogenesis, etc.), we thought it would be more effective to restrict the range of the subjects treated and instead to concentrate discussion on the general theme of 'Structure-Activity Relationships'. This type of procedure seems justified to us for several reasons. On the one hand, other international meetings have focussed on progress in radioimmunoassay methods and, on the other, in our knowledge concerning the metabolic influence of hormones, but the greatest progress has surely been seen in recent years in the realm of hormonal structure.

Clinical and immunological research has allowed advances to be made in our knowledge of the structure of hormone glycoproteins and has provided evidence of their subunits. The sequence of the smaller polypeptides has been established and their synthesis effected.

It has been possible to study their structural analogues. Furthermore, high-resolution physical techniques (NMR) are providing extremely accurate information on the involved structure of these hormones.

Finally, a better knowledge of the structures of the hormones and their analogues is allowing us to approach the problem of their sites of action and receptors and to define certain of their characteristics. These topics seem to us to comprise a homogeneous topic which is undergoing rapid expansion and is of the greatest interest to biochemists and endocrinologists.

We hope that this meeting, thanks to its mode of organization, will allow the distinguished scientists taking part to assess attainments made in this particularly interesting field.

Lastly we should like to express our particular thanks to Professor S. A. Berson for his unfailing and invaluable help in drawing up the scientific program.

A. NIZET
H. VAN CAUWENBERGE

INTRODUCTION

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The present Symposium, organized to discuss the relationship between the structures of the peptide hormones and their activities in biologic systems, considers two quite disparate aspects of hormonal specificity. This dichotomy is reflected in the format of the Symposium, which distinguishes individual sessions assigned solely to the 'chemical' aspects or to the 'immunologic' aspects of structure-activity relationships. The terms are not completely descriptive since both aspects must be related to the chemical characteristics of the hormone as well as to those of the system with which the hormone reacts. A goal to which both types of studies aspire is an understanding of this reaction at the molecular level.

The sessions devoted to the so-called chemical aspects are concerned with the activity of the hormone *qua* hormone, *i.e.* with those specific actions of the hormone on cellular systems that eventually find expression in a physiologic effect, whereas those on the immunologic aspects are involved with the hormone only as an immunogen or as a reactant with antibodies, *i.e.* not with the hormonal role of the hormone.

'Hormonal activity' must be considered on several levels, the first of which is the initial reaction of the hormone with its target cell. The steroidal and thyroidal hormones, perhaps owing to their high lipid-solubility, are able readily to pass through the cell membrane and are then, at least in some cases, complexed to a cytoplasmic protein, eventually, again at least in some cases, to be carried into the nucleus. The structural requirements for membrane transport are yet to be studied but specificity is more likely to be a function of cytoplasmic binders and of later steps in intracellular activity. In contrast, all evidence to date suggests that peptide hormones, like most of the biogenic amines do not penetrate to the cell interior but react with a receptor on the plasma membrane. The binding of isotopically labeled hormones to cell receptors had its beginnings some years ago. Stadie and his collaborators (Stadie *et al.*, 1949) had concluded that insulin becomes bound to tissues from observations that the isolated rat diaphragm, only briefly immersed in a solution of insulin and then washed repeatedly, later showed insulin effects in the form of glycogen synthesis. Early attempts to quantify this binding with ^{131}I-insulin (Stadie *et al.*, 1952) were frustrated both by the tendency of insulin, in common with many other peptide hormones, to adsorb non-specifically to glassware and to the tissues (Newerly and Berson, 1957) and by the use of excessive hormone concentrations. Contemporary studies, using membrane preparations obtained from fragmented cells and much improved methodology are described in this Symposium.

Consideration of the requirements for binding of peptide hormone to membrane receptor must clearly include both hormone and receptor, the implication being that there exists the possibility that receptors for the same hormone may have significant structural variations among different tissues in the same animal as well as among similar tissues of different species. For the latter, it has already been shown that whereas arg^8-vasopressin (AVP) has greater antidiuretic activity than lys^8-vasopressin (LVP) in species that make the former hormone (Van Dyke *et al.*, 1956), LVP is as potent or even more potent in the pig, which makes LVP (Munsick *et al.*, 1958). Comparative studies of the affinities and activities of hormonal congeners for different target tissues in the same animal are required; it has yet to be excluded that even tissues indifferent to the effects of a hormone can bind it.

That the structural requirements for binding are not altogether identical with those for other hormonal activities is evident from the existence of antagonists as well as agonists

among the analogs and congeners of hormones. Presumably, any such antagonistic analogs with little or no agonistic activity are capable of binding to the receptor site but lack the ability to activate the mechanism responsible for initiating the chain of events leading to expression of hormonal activity. On a second level of 'hormonal activity' we must then be concerned with 'activating sites' as well as 'binding sites' of the hormone molecule. These two activities can be distinguished by using labeled hormones and analogs together with the unlabeled forms in competitive inhibition studies to explore the structural requirements for binding and by measuring the biochemical effects to elucidate the requirements for 'activation'. Although the mechanism by which the hormone-receptor complex activates the next step remains elusive, the brilliant achievements of Sutherland and his colleagues have provided, for many hormones, a means to demonstrate that activation has taken place. The intimate association of adenyl cyclase with the membrane allows the production of 3′,5′ cyclic AMP in membrane preparations to serve as evidence of activation.

In the case of a hormone such as insulin, which tolerates little alteration without loss of biologic activity, the inference seems justified that the activating site is somewhat removed from the binding site, since it appears unlikely that the entire molecule is intimately involved in the binding reaction. Studies on the binding of biologically inactive fragments of insulin should clarify these relationships. However, very short peptide sequences are biologically active, *e.g.* the N-terminal tetrapeptide amide of gastrin, which reproduces all the biologic effects of the parent hormone, and the tripeptides of the hypothalamus possessed of thyrotropin-stimulating and melanotropin-inhibiting activity. The clear dissociation between anionic and esteratic sites on acetylcholine esterase and the abundant examples of cholinergic and adrenergic blocking agents that bind, but do not activate, should quickly remove any hesitation in granting the possibility of separate and independent binding and activating sites on the smallest of the peptide hormones.

Another level of hormonal activity that demands attention is that concerned with protein synthesis. The ability of many peptide hormones to produce their well-known effects within minutes, even seconds, the ability of cyclic AMP or phosphodiesterase inhibitors to reproduce these effects and the inability of inhibition of RNA and protein synthesis to block these effects suggests that we need look no further than activation of the cyclase system to explain these immediate effects of the peptide hormones, which are presumably all mediated by activation of existing inactive enzymes. (Some of the effects of insulin are explicable in terms of inhibition, rather than activation, of adenyl cyclase.) However, a late increase in protein synthesis is a frequent consequence of the action of peptide hormones. Is this too to be attributed to cyclic AMP? Steroids commonly induce enzyme synthesis, in contrast to enzyme activation, and there is evidence that their influence is at the transcriptional level; the nuclear localization of steroidal hormones would be consistent with a derepression-type mechanism for these effects. Thyroxin, which also induces enzyme synthesis, has recently been reported to localize in the nucleus of certain target cells (Dratman and Buck). There is increasing evidence that cAMP as well as hormones (epinephrine and glucagon) known to activate the cyclase system can induce hepatic enzymes among which have been reported, phosphopyruvate carboxylase (Yeung and Oliver, 1968), tyrosine aminotransferase (TAT) (Wicks, 1968) and serine dehydratase (Jost *et al.*, 1961). Although there are suggestions that steroids induce enzymes through cAMP (Szego and Davis, 1967; Sharp *et al.*, 1968) it would appear that cAMP-induced and steroid-induced enzyme synthesis operate through different and independent mechanisms, since an additive response is seen when epinephrine is added to steroid but not when added to cAMP (Wicks, 1968) and since only steroids induce TAT synthesis in Buffalo rat hepatoma cells, in which adenyl cyclase could not be detected and negligible amounts of cAMP were measured even after fluoride (Granner *et al.*, 1968). To exclude the role of cAMP as the 'second messenger' of Sutherland in the enhancement of protein synthesis by peptide hormones, it would be necessary to demonstrate that the nucleotide is incapable of reproducing the effects of a given peptide hormone on protein synthesis in tissues that exhibit the initial hormone-like effect in response to cAMP.

For several reasons it seems unlikely that cAMP is the only second messenger for all effects of peptide hormones. In cells whose cyclase system is activated by several hormones the type of protein synthesized would no longer be specifically controlled by an individual hormone. Furthermore, insulin seems to inhibit the cyclase system and insulin stimulates

protein synthesis. Finally, there are certain effects of peptide hormones that seem not to be influenced by changes in intracellular cAMP; for example, when the antilipolytic effect (presumably effected through reduction of intracellular cAMP) of insulin is counteracted by epinephrine (which increases intracellular cAMP), the effect of insulin on glucose transport in the fat cell is not altered (see Sutherland and Robison, 1969). Sutherland and Robison (1969) have suggested that insulin may stimulate another second messenger. If, then, we continue to regard the site of action of peptide hormones to be localized to the plasma membrane we must postulate the existence of additional membrane-linked systems that, like adenyl cyclase, are activated by the hormone-receptor complex, and must consider whether the structure of the 'activating site' of the hormone for these systems is the same as that for the cyclase system. To begin to explore this question it will be necessary first to establish cyclase-independent effects of peptide hormones and then to examine the structural-requirements of the hormones exerting these effects. Rat diaphragm has classically served as a test tissue for insulin action on glucose uptake and protein synthesis, both of which may be cyclase-independent. The Buffalo rat hepatoma cell, which appears to lack adenyl cyclase and cAMP and is unresponsive to cAMP, at least in certain respects (Granner *et al.*, 1968), should be another useful tissue to test.

Complete pictures of hormone-receptor interaction and activation mechanism must include not only a description of the linear sequence of amino acids of the peptide hormones and of the chemical groups of the membrane participating in these processes but also three dimensional views of the structures of the reactant in their 'resting' state and of the complexes, revealing the deformations suffered in the reactions. Studies on the sequences involved in binding reactions have classically been pursued with fragments of hormone molecules and with various analogs containing substitutions, altered or added side groupings, and deletions or intercalations in the sequence. Chemical alterations of the peptide not only change the site of alteration itself but also might affect the three dimensional structure in such a way as to change the conformation at a site distant from the original alteration. It is therefore risky to conclude from the diminished reactivity of an altered molecule that the site of alteration itself is necessarily involved in the reaction site. Furthermore, although present dogma holds that the three dimensional structure is determined by primary structure (amino acid sequence), *i.e.*, that one particular configuration provides such a low energy state by comparison with all others that the probability of any other configuration is negligible, there exists in the records an observation not explicable within the confines of this dogma; namely, certain insulin antisera are capable of distinguishing different species' insulins with reportedly identical amino acid sequences (Berson and Yalow, 1961, 1966). For these reasons as well as others conformational studies are essential to a full understanding of structure-activity relationships and specificity of hormone action.

The conformations of some of the peptide hormones in their uncombined state are discussed in the present Symposium but we are as yet very far removed from any picture of receptor sites for peptide hormones. Solubilization of such sites would accelerate progress immeasurably; perhaps this will have been accomplished by the time of the Symposium.

Finally, while it should be reiterated that the immunogenicity and immunoreactivity of peptide hormones are of little direct interest to an understanding of structural requirements for hormonal activity, immunologic studies are capable of providing information about the structure of peptide hormones and of contributing to refinements in radioimmunoassay, which may play a role in elucidating strictly hormonal functions. Evidence indicating that human insulin reacts more like porcine than bovine insulin in human insulin antisera (Berson and Yalow, 1959) preceded the elucidation of the structure of human insulin and the demonstration that human and porcine insulin differ in only a single amino acid (Nicol and Smith, 1960). Deductions on conformational changes may also be derived from immunologic studies. General aspects of immunologic versus biologic specificity are discussed elsewhere in this volume (see pp. 38-47).

Questions raised here, and many others, will be the subjects of fruitful discussion in this Symposium. It is fitting to applaud our hosts for their selection of Structure-Activity Relationships as the subject of the present Symposium, since this subject must ever remain of the most fundamental interest in any field of biologic investigation and is presently only in its infancy with regard to the peptide hormones. Great percipience is not demanded for the