PEPTIDE HORMONES

1969 Cuthbert: *Calcium and Cellular Function*
1970 Aldridge: *Mechanisms of Toxicity*
1971 Rabin and Freedman: *Effects of Drugs on Cellular Control Mechanisms*
1972 Rang: *Drug Receptors*
1973 Callingham: *Drugs and Transport Processes*

BIOLOGICAL COUNCIL
The Co-ordinating Committee for Symposia
on Drug Action

PEPTIDE HORMONES

Edited by
J. A. PARSONS, M.A., B.M., B.Ch.

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Co-ordinating Committee for Symposia on Drug Action

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in London
at The Middlesex Hospital Medical School*

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and
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G. T. Young

The Amino Acids Found in Natural Proteins and Polypeptides

The conventional abbreviation and molecular weight is shown beneath each name. To calculate the molecular weight of a protein chain, subtract one molecule of water (18.015) for each peptide bond formed.

Glycine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{H} \\ \\ \text{COOH} \end{array}$	Valine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_2\text{N}-\text{CH}-\text{CH}-\text{CH}_3 \\ \\ \text{COOH} \end{array}$
Gly 75.07		Val 117.15	
Alanine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_3 \\ \\ \text{COOH} \end{array}$	Isoleucine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_2\text{N}-\text{CH}-\text{CH}-\text{CH}_2-\text{CH}_3 \\ \\ \text{COOH} \end{array}$
Ala 89.09		Ile 131.18	
Aspartic Acid	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}-\text{OH} \\ \quad \quad \quad \\ \text{COOH} \quad \quad \text{O} \end{array}$	Leucine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}-\text{CH}_3 \\ \quad \quad \quad \\ \text{COOH} \quad \quad \text{CH}_3 \end{array}$
Asp 133.10		Leu 131.18	
Glutamic Acid	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{C}-\text{OH} \\ \quad \quad \quad \quad \\ \text{COOH} \quad \quad \quad \text{O} \end{array}$	Methionine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_3 \\ \\ \text{COOH} \end{array}$
Glu 147.13		Met 149.22	
Pyroglutamic Acid	$\begin{array}{c} \text{HN}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{C}-\text{OH} \\ \quad \quad \quad \\ \text{O}-\text{CO} \quad \quad \text{O} \end{array}$	Phenylalanine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}_6\text{H}_5 \\ \\ \text{COOH} \end{array}$
Glu		Phe 165.19	
Asparagine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}-\text{NH}_2 \\ \quad \quad \quad \\ \text{COOH} \quad \quad \text{O} \end{array}$	Tyrosine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}_6\text{H}_4-\text{OH} \\ \\ \text{COOH} \end{array}$
Asn 132.12		Tyr 181.19	
Glutamine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{C}-\text{NH}_2 \\ \quad \quad \quad \\ \text{COOH} \quad \quad \text{O} \end{array}$	Tryptophan	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}=\text{C}-\text{C}_6\text{H}_4 \\ \quad \quad \quad \quad \\ \text{COOH} \quad \quad \text{HC} \quad \text{N} \end{array}$
Gln 146.15		Trp 204.22	
Proline	$\begin{array}{c} \text{H}_2 \quad \text{H}_2 \\ \quad \\ \text{C}=\text{C} \\ \quad \\ \text{HN}-\text{CH}-\text{CH}_2 \\ \\ \text{COOH} \end{array}$	Histidine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{C}=\text{CH} \\ \quad \quad \quad \\ \text{COOH} \quad \quad \text{HN} \quad \text{N} \\ \quad \quad \quad \\ \quad \quad \quad \text{C} \\ \quad \quad \quad \\ \quad \quad \quad \text{H} \end{array}$
Pro 115.13		His 155.16	
Serine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{OH} \\ \\ \text{COOH} \end{array}$	Arginine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}-\text{C}=\text{NH}_2 \\ \\ \text{COOH} \end{array}$
Ser 105.09		Arg 174.21	
Threonine	$\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_2\text{N}-\text{CH}-\text{CH}-\text{OH} \\ \\ \text{COOH} \end{array}$	Lysine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2 \\ \\ \text{COOH} \end{array}$
Thr 119.12		Lys 146.19	
Cysteine	$\begin{array}{c} \text{H}_2\text{N}-\text{CH}-\text{CH}_2-\text{SH} \\ \\ \text{COOH} \end{array}$		
Cys 121.15			

Foreword

The papers in this book have been expanded and updated from reviews given at a two-day symposium, the main aim of which was to assist the exchange of information between those working with the very varied peptide chains which are secreted as endocrine messengers. We hope that publication will further this aim, giving to a wider audience an integrated account of the biological significance and diagnostic and therapeutic potential of these hormonal peptides, more than 30 of which are now known. Within the space available, some contributions to the discussion have also been included when they raised points which seemed to the editor of general interest and were not fully covered in the original presentations.

The peptide hormones circulate in the blood at concentrations measured in picograms (10^{-12} g) or nanograms (10^{-9} g) per ml and, because of their similarity to the major structural and enzymatic proteins of the body, were particularly difficult to study before the introduction of modern methods for non-denaturing separation and highly sensitive measurement and analysis. Once these became available, knowledge advanced so fast that the field has tended to become specialised and fragmented. The Symposium was therefore designed around brief reviews of ten major peptide hormone groups and reviewers were asked to cover as far as possible topics in a pre-circulated checklist. This list (Appendix 1), was designed to bring out similarities and differences between the various peptide-secreting systems and may be useful for classifying information on new hormones as well as old.

Although knowledge of the various hormone systems has so far developed more or less independently, it is clear that each requires synthesis of hormone precursor and receptor molecules and that the evolution of present function has also required highly specific processes of cleavage and translocation to achieve activation, storage, secretion and destruction of the messenger molecules. A more profound understanding of physiology is likely to result from comparative studies of the way in which each step has come to serve an appropriate control function.

Many peptide hormones still fall into the category of substances whose biological activity cannot be precisely characterised by chemical or physical means and are therefore assayed in units defined by a biological standard. Since the numerical value of such a unit is essentially arbitrary when assigned

Table 0.1 Biological activities of some peptide hormones. Hormones are listed in the order of discussion in this book. Estimates of specific activity are imprecise for the very reasons which make bioassay necessary and are subject to adjustment in the light of changes in isolation procedure or assay technique. The (quite different) requirements for radioimmunoassay standards are discussed by Bangham and Cotes (1971) and in the 26th ECBS Report (1975)

Hormone	Mol. wt.	Current standard by which biological unit is defined*	Specific activity reported for purest preparation (U/mg of hormone named in column 1)	Reference for column 4†
(1)	(2)	(3)	(4)	(5)
Insulin (porcine/bovine)	5764 (porcine)	4th IS (porcine/bovine)	28	Root <i>et al.</i> (1972); Schlichtkrull <i>et al.</i> (1975)
Glucagon (porcine)	3483	1st IS (porcine)	1 (by definition)	Annable <i>et al.</i> (1974)
Parathyroid hormone (bovine)	9562	1st IRP (bovine)	2500	Parsons and Potts (1972)
Calcitonin (porcine)	3607	1st IRP (porcine)	200	Brewer <i>et al.</i> (1968);
Calcitonin (human)	3421	MRC Res. Std. A (human)	120	Riniker <i>et al.</i> (1968); (porcine standard);
Calcitonin (salmon)	3435	1st IRP (salmon)	2700	Keutmann <i>et al.</i> (1970)
Oxytocin	1010	3rd IS (bovine pituitary)	450	Boissonnas <i>et al.</i> (1961)
Vasopressin (Arg ⁸)	1087	3rd IS (bovine pituitary)	400	Boissonnas <i>et al.</i> (1961)
Renin (porcine)	35 000 (approx.)	MRC Res. Std. A (porcine)	780	Haas <i>et al.</i> (1953)
Prolactin (ovine)	22 554	2nd IS (ovine)	28	Frantz <i>et al.</i> (1972)
Growth hormone (human)	22 005 (human)	1st IS (bovine) (no bioassay standard for human hormone yet available)	about 2	Mills <i>et al.</i> (1969)
Thyroid stimulating hormone (human)	28 000 (approx.)	MRC Res. Std. A (human)	about 20	Condliffe (1963) (bovine standard)
Corticotrophin (porcine)	4567	3rd IS (porcine) (see caution on S-C assays—this vol p. 257)	100 (by definition)	Bangham <i>et al.</i> (1962)

Table 0.1 (*cont.*)

Hormone	Mol. wt.	Current standard by which biological unit is defined*	Specific activity reported for purest preparation (U/mg of hormone named in column 1)	Reference for column 4†
(1)	(2)	(3)	(4)	(5)
Follicle stimulating hormone (human pituitary)	37 000 (approx.)	1st IRP (human pituitary powder)	14 000	Roos (1968)
Luteinising hormone (human pituitary)	29 000 (approx.)	1st IRP (human pituitary powder)	5000	NIBSC (1975)
Chorionic gonadotrophin (human)	36 700 (approx.)	2nd IS (human)	10 000–15 000	Canfield <i>et al.</i> (1976)

*IS = International Standard (designation reserved for hormones established in international commerce). IRP = International Reference Preparation (designated by Expert Committee on Biological Standardisation of World Health Organisation). MRC = Medical Research Council (London). All standards available from National Institute for Biological Standards and Control, Holly Hill, London NW3 6RB, England.

†Assays cited in this column were not necessarily carried out with the standards named in Column 3.

to a first standard (although sometimes historically related to physiological response in a 'average' animal), unfamiliar figures constitute one of the stumbling blocks to the understanding of biologists reading about a variety of hormones. Approximate mass equivalents are therefore given whenever units are mentioned throughout the book, and molecular weights are liberally included. For convenience, these data are collected in table 0.1, which lists the standards or reference preparations of peptide hormones distributed on behalf of the World Health Organisation by the National Institute for Biological Standards and Control.

This book assumes rather more knowledge of peptide chemistry than may be possessed by all biologists, and a word is due on the conventions adopted. The chemical structures and molecular weights of the amino acids found in natural proteins and peptides are shown in the frontispiece, and their shapes are also illustrated in the review of their characteristics by Rudinger (figure 1.1). In consequence of the head-to-tail union of amino acids and the unsymmetrical nature of the peptide bond, each element of a peptide chain has direction. This is conventionally shown by numbering and drawing from the amino-terminus on the left. The terminal amino group may or may not be shown (as H— or NH₂—), but in any case is assumed to be free (unsubstituted) unless it is present in a ring (amino-terminal prolyl or pyroglutamyl) or shown as acylated (e.g. CH₃CONH— or Ac—). Similarly, the carboxyl terminus may or may not be shown at the right-hand end of the chain (—OH or —COOH) but is also assumed

to be free unless shown as —NH_2 , which here indicates an amide group (no free carboxyl terminus) and not an amino group. It may also be helpful to know that *in vivo* biosynthesis begins at the amino terminus and therefore proceeds in the direction of conventional numbering and that the Edman degradation, now used in so many structural studies, removes residues serially from the amino terminus. On the other hand, most present methods of solid state synthesis depend on attaching a carboxyl terminal amino acid to a resin and proceed *towards* the amino terminus. Thus it is easy to subdivide a synthesis so as to prepare a family of peptides modified at the amino terminus, but each modification at the carboxyl terminus requires a fresh start.

It is a pleasure to acknowledge the generous help from so many people which made the Symposium possible. The major investment in peptide endocrinology in the USA and Switzerland is reflected in the fact that eleven of our speakers came from these countries. This was only made possible by generous financial support from the Wellcome Trust, Sandoz, Ciba-Geigy and Imperial Chemical Industries and because the expenses of accommodating speakers were almost entirely met by the Ciba Foundation. The reviewers cooperated splendidly in their difficult assignment, only one or two throwing up their hands in despair at the thought of painting the outlines of their speciality clearly in no more than half an hour—and even these bravely agreed to complete their task before publication. The hard work and experience of Miss G. M. Blunt were invaluable and members of the staffs of NIMR and NIBSC provided indispensable facilities and assistance before, during and after the meeting (including my own group, who have also cheerfully put up with the amount of time I had to spend on it).

It is with great sadness that I record the death on 30 April 1975 of Professor J. Rudinger, after a short illness at the age of only 51. We are very fortunate to have his clear and original summary of the characteristics of the amino acids (which he typically deprecated on the grounds that all the information in it was already published elsewhere). His great mind and warm personality will be sadly missed by his many colleagues and friends.

J. A. P.

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List of Speakers

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- G. F. Cahill, Jr., Director, Joslin Research Laboratory, Boston, Massachusetts, USA
- R. E. Canfield, College of Physicians and Surgeons of Columbia University, New York, USA
- P. Cohen, Department of Biochemistry, University of Dundee, Dundee, UK
- N. Fleischer, Department of Medicine, Albert Einstein College of Medicine, Yeshiva University, New York, USA
- A. G. Frantz, College of Physicians and Surgeons of Columbia University, New York, USA
- M. I. Grossman, Veterans Administration Center, Los Angeles, California, USA
- R. Hall, Department of Medicine, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK
- R. J. B. King, Imperial Cancer Research Fund, London, UK
- *A. H. Kissebah, St. Mary's Hospital Medical School, London, W2
- J. Landon, Department of Chemical Pathology, St. Bartholomew's Hospital Medical College, London, UK
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- H. D. Niall, Department of Biochemistry, Howard Florey Institute, University of Melbourne, Australia.
- J. A. Parsons, National Institute for Medical Research, Mill Hill, London, UK
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*Invited contributor to discussion.

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although major, energetically disfavoured structural rearrangements are admittedly unlikely.

SIGNIFICANCE

In a given molecule some amino acids or sequences obviously owe their 'significance' to their inclusion in the pattern which is directly involved in recognition by, and binding to, the receptor. However, the fact that the existence of this pattern is dependent on a conformation stabilised by intramolecular interactions, as discussed above, implies that other amino acids or sequences contributing to this conformational stability will be no less 'significant' for the biological activity of the molecule. Even more generally, sequences contributing to those properties of the peptide which affect its transport and distribution, metabolic transformations, binding to non-receptor sites, etc., may significantly modify its biological activity. In defining the relation between sequence and activity it is necessary to take into account all these contributions, and not merely those directly involved in receptor binding*.

Two separate events may be conceptually distinguished in hormone-receptor interaction: binding (recognition) and stimulation (initiation of the signal which eventually leads to the observed response). In some of the models proposed to account for hormone action, the process of stimulus generation is actually identical with the process of binding. These are variants of the 'allosteric' model (Monod, Changeux and Jacob, 1963), which have in common the assumption that binding of hormone at one site modifies the activity of a second, topologically distinct site on the same molecule, probably by inducing a conformational change. In other models (the 'participation' type; Rudinger, Pliška and Krejčí, 1972) binding and stimulus generation are two distinct molecular events.

The properties of a series of oxytocin analogues modified at the tyrosine residue (position 2) illustrate a possible experimental approach to this problem and favour a 'participation' model for oxytocin. As shown in figure 1.4, replacement of the hydroxyl group of this tyrosine by various substituents leads, in a graded manner, to loss of oxytocin-like activity and to the appearance of inhibitor properties. Yet the binding affinity, determined from pharmacological parameters (pD_2 and pA_2), is practically the same for the whole series of analogues, suggesting that the region of the molecule around position 2 is involved in stimulus generation but contributes little to binding (see Rudinger *et al.*, 1972). Moreover, we have recently found that substitution *ortho* to the tyrosine hydroxyl group—for example, by iodine or methyl—also gives rise to inhibitors: evidently the hydroxyl group is

*Obviously, these various functions need not and, in general, will not be attributable to separate and distinct sequences, but particular amino acids, sequences or topochemical regions may participate in several of them.