Gut Hormones

Edited by

S. R. Bloom

Gut Hormones

Edited by

STEPHEN ROBERT BLOOM, M.A., M.B., B.Chir., MRCP,
Senior Lecturer and Honorary Consultant in Clinical Endocrinology,
Department of Medicine,
Royal Postgraduate Medical School,
Hammersmith Hospital,
London, W12 0HS.

Foreword by

MORTON I. GROSSMAN,
Veterans Administration Wadsworth Hospital Center,
Los Angeles, California, USA.

CHURCHILL LIVINGSTONE
EDINBURGH LONDON AND NEW YORK 1978

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by
Longman Inc., 19 West 44th Street, New York,
N.Y. 10036 and by associated companies,
branches and representatives throughout
the world.

ISBN 0 443 01662 3

British Library Cataloguing in Publication Data

Gut hormones.
1. Gastrointestinal hormones
I. Bloom, Stephen Robert
612'.33 QP572.G35 77–30699

Printed in Great Britain by Lowe & Brydone, Thetford, England.

Foreword

Interest in gut hormones is no longer limited to gastroenterologists. The first fraternization between gut endocrinologists and general endocrinologists came a few years ago with the recognition that gut hormones participate in regulation of release of the hormones of the pancreatic islets and that several peptides are found in both the gut and the islets. Very recently, a veritable flood of discoveries has shown that in many instances the same or similar peptides are present in gut endocrine cells, in endocrine cells in other organs, and in central and peripheral nerves. Already, seven peptides have been shown to be present in both gut and brain. Quite understandably, this has aroused the keen interest of investigators in many fields. Physiologists of the gut and of the brain are no longer strangers. The concept is rapidly evolving that the body has a single system of chemical messengers comprising an interrelated group of peptides and amines. The same or similar chemical messengers may be utilised for neurocrine, paracrine, and endocrine modes of transmission. The concept is breathtaking in its simplicity and audacity. The major mechanisms for coordinating bodily activities can now be viewed as a unified system.

Advances in gut hormones are occurring so rapidly and are so diverse that even the experts need frequent comprehensive reviews. Add to this the interest of those outside the field of gastroenterology and it is easy to understand why the field commands such attention.

This book provides a comprehensive and systematic summary of the state of the art in all aspects of this rapidly developing field. This includes, among others, the chemistry and synthesis of the gut peptides and their many heterogeneous forms, the assessment of the physiological and pathological role of each peptide, the problems involved in perfecting radioimmunoassays for each peptide, and the assignment of a cell of origin for each peptide.

MORTON I. GROSSMAN
Veterans Administration Wadsworth Hospital Center,
Los Angeles, California, USA.

Preface

The influence of gut hormones in alimentary and metabolic disorders is now well recognised. The considerable difficulties in investigating the alimentary tract makes the diagnostic potential of gut endocrinology extremely attractive. Indeed to help with diagnosis and management several countries have set up regional assay centres where clinicians can send samples for measurement of gut hormones. The importance of such hormone measurement has been illustrated recently in the investigation of coeliac disease. In this condition, release of duodenal hormones, such as secretin, are blocked by the mucosal disease but compensatory changes in the lower small intestine lead to a very great increase in the ileal hormones, for example enteroglucagon. A "gut hormone profile" thus gives considerable information on the nature of the abnormality, yet requires from the patient only a simple venepuncture. An exciting clinical development in another direction are new studies of the pathology of the endocrine cells in human mucosa biopsies which were taken during routine endoscopic examination in the outpatient clinic.

On the scientific front the investigation of gut endocrinology has led to fundamental advances in our understanding of physiology. A triple control system, neurocrine, paracrine and endocrine has been demonstrated in the gut. The old concepts of a hormone are being challenged by finding that many of the hormonal peptides also appear to act as neurotransmitters, in the brain, for example, influencing cerebral functions such as temperature control, pain and mood.

The stimulus to produce this book was a meeting held in Lausanne, Switzerland in the Summer of 1977 to celebrate the 75th Anniversary of the discovery of the first hormone, secretin. It was clear that the field was developing so rapidly that even conventional journal articles were lagging far behind. Thus the coming together of all the relevant experts and the scientists responsible for the recent great advances provided an unrivalled opportunity to prepare a comprehensive and totally up-to-date book summarising the field..

I am indebted to each of the contributors both for their superb chapters and their remarkable promptitude. The untiring assistance of my colleagues on the Organising Committee and the Editorial Advisory Committee is most gratefully acknowledged. Nothing would have been possible without the cheerful efficiency of my secretary Miss Alicia Barker and also the encouragement of my wife Margaret.

STEPHEN BLOOM

October 1977

Support was received from the Dr. Auguste Widmer Foundation

Contents

INTRODUCTION

1. Gut hormone overview

S. R. Bloom and J. M. Polak
Royal Postgraduate Medical School, London W12 0HS, U.K.

Introduction

The subject of gut endocrinology is a venerable one and it has grown up with the century. Beginning with the discovery of secretin in 1902 and gastrin in 1905, progress has been steady if unspectacular until the last decade. As students we were taught that the system was simple. Gastrin controlled gastric acid, secretin controlled pancreatic bicarbonate, cholecystokinin-pancreozymin controlled gall bladder contraction and pancreatic enzyme output. Physiologists and morphologists, however, knew that there were many unexplained control systems and a large number of different endocrine cell types whose product was not yet recognised. In comparison with other branches of endocrinology investigation of the hormones of the gastrointestinal tract was impeded by the fundamental difficulty of investigating a diffuse endocrine system. Contrariwise the glandular endocrine system had been easy. Extirpation of an endocrine gland gave rise to the deficiency syndrome and injection of dried gland extract produced the symptoms of excess. These endocrine cells were formed into glands either because they responded to a blood-borne stimulus and so required to sample blood only from a single supplying artery, or alternatively, they responded to a discreet single nerve. By contrast the diffuse endocrine system is designed to produce an integrated signal in response to a diffuse and discontinuous stimulus. Thus the endocrine cells of the gut respond to such uneven stimuli as lumps of ingested food. An integrated response is required that correctly assesses the total quantity of carbohydrate, protein or fat ingested so that the appropriate digestive and metabolic responses can be called into play. Studying the diffuse endocrine system is difficult. The cells are widely scattered through a great bulk of tissue and usually many different endocrine cell systems overlap. Thus extirpation of a single cell type is impossible and tissue extracts contain several different hormonal substances in a vast bulk of interfering protein and peptide material. Progress in understanding gut endocrinology was therefore difficult and slow. The major factor that has recently so greatly accelerated its investigation has been the development of enormously improved chemical purification techniques. The discovery of ion exchange chromatography, gel chromatography, affinity chromatography, isoelectric focussing etc.,

now enables the chemist to extract a specific hormonal peptide with relative ease. Thus the finding of new peptide hormones has recently been very successful and we now have a number for which no known physiological role has yet been found. Indeed the rate of accretion of new peptide hormones has been so bewildering that many workers have been tempted to suggest that some of these substances may have no physiological role. The fundamental principle behind the theory of evolution is the process of natural selection. Thus any system not essential for survival is selected against and gradually removed. As there is no evidence for the disappearance of any of the new hormonal peptides across the mammalian species, one must assume that they are, indeed, essential for our survival. Failure to discover their physiological role is therefore more a reflection of our own scientific ineptitude.

Use of antibodies

Following the identification and isolation of a new active peptide, the next stage in its investigation is to develop an assay system capable of detecting it in low concentrations in complex situations, for example in plasma. Its exact localisation in the tissues of the body and its dynamic response to stimuli can then be investigated. Equally important is the discovery of its cellular origin. The fantastic, and almost unbelievable, ability of the immune system of higher vertebrates to produce protein antibodies which combine with enormous avidity and high specificity to any peptide appropriately administered enables us to achieve these objectives. These specific antibodies are used in radioimmunoassays to detect fmol quantities of a hormone, while being unaffected by many similar peptides. Similarly immunocytochemical techniques use antibodies to pick out the single cells producing a given hormone from a vast mass of tissue containing many other different types of endocrine cells. Both techniques have thus gained wide acceptance and have now become the everyday working tools of gut endocrinology. Perhaps, however, familiarity has begun to breed contempt. The precise quantitative reactions of an antibody with its antigen is now being taken for granted and often the numerical result of such a reaction is quoted to two or three decimal places. The antigen binding sites of antibodies are, however, not immutable. Steric changes in the gamma globulin molecule, and thus in its binding site, are quite easy to produce by small environmental alterations, for example slight changes in protein or ionic concentration. Such changes grossly alter the quantitation of the antibody antigen reaction. Gut hormone meetings are frequently enlivened by acrimonious exchanges between radioimmunoassayists concerning the errors of their respective assays, as the basic properties of antibodies are not well understood. These problems are discussed in greater detail in the section on radioimmunoassay.

Feyrter drew attention in 1938 to the presence of numerous cells diffusely distributed throughout the body which failed to take up conventional stains. These clear cells, which were apparent in the mucosa of the gut, had the special property of reducing

silver salts (argentaffin) or at least of taking up silver salts which could subsequently be reduced by the addition of a reducing agent (argyrophil). The dense silver staining of these cells thus made them easy to pick out from the other epithelial cells of the mucosa. Feyrter proposed that these cells exerted local hormonal control (paracrine). Later studies with the electron microscope demonstrated these cells to have numerous secretory granules and thus confirmed their endocrine nature. It became apparent, however, that these secretory granules showed marked differences from cell to cell. There thus developed an ultrastructural classification around the granule type, with each different cell being given a code letter. The first general meeting of ultrastructural morphologists took place in Wiesbaden in 1969 and an international classification was agreed. Subsequently, several new cells have been discovered and other cell types subdivided. A further international meeting of experts was therefore held in Lausanne, Switzerland, in June 1977 and the amended classification is given in Chapter 10. In 1966 Pearse put forward the unifying hypothesis that all the peptide producing cells of the gut and pancreas belonged to a much larger group of cells having many cytochemical, ultrastructural and functional characteristics in common. To characterise this group the acronym APUD was invented, based on some of the more important common staining characteristics. These APUD or endocrine cells of the gut can be divided into two types, open and closed. The open cells have a direct connection with the gut lumen by means of microvilli and their secretory granules tend to be localised towards the cell base. In contrast the closed type of cell does not show any connection with the lumen and tends to be round in shape rather than elongated. One may speculate that these later cells respond more to the local tissue environment than to food stimuli in the gut lumen.

Neither ultrastructural nor the classical cytochemical techniques yield any firm information as to which hormone is being secreted by a given cell. Use is made of the specificity and great avidity of antibodies, as mentioned above, to determine this fact. Immunocytochemistry has become one of the most reliable tools for picking out and studying the cell producing a particular hormone. In the development of this technique three main problems were overcome

1. Fixation
2. Specificity
3. Quantification

Clearly hormones are very water soluble and therefore the product of an endocrine cell in a tissue section would be rapidly washed away unless adequately fixed. The act of fixation, however, which involves covalent cross linking is very liable to alter the antigenicity of the hormone so that it is no longer recognised by specific antibodies. The development of two cross-linking agents, benzoquinone and diethylpyrocarbonate, which cause minimal change in peptide structure, has largely solved the problem. On the other hand, the second problem, the specificity of immunocytochemical staining reactions, still requires considerable care. There are two main aspects. Firstly certain tissues, particularly when badly fixed, may absorb gamma globulin non-specifically. This artefact can be readily detected by the routine