INTRODUCTION

The rate at which a field of experimental biology is currently advancing can be gauged, to a large extent, by the range of techniques that can be applied successfully to its central questions. Nowhere is this more evident than in the field of stimulus–secretion coupling – the cascade of events in cells leading to the controlled discharge of a chemical (primary messenger) in response to a defined input signal. The present volume provides adequate evidence of the progress achieved by combining the latest technical advances in biochemistry, biophysics and molecular biology with the more traditional physiological and ultrastructural approaches to the study of secretion.

Detailed analysis of the secretory process requires knowledge of the subcellular organization of secretory terminals and precise localization of key molecular components. As illustrated in the following pages, a variety of vertebrate secretory cell types, including cells of the paraventricular hypothalamus, the anterior, posterior and intermediate pituitary lobes, the adrenal medulla, mast cells and pancreatic cells, together with invertebrate neurosecretory organs, and purified nerve terminal fractions (secretosomes and synaptosomes) have yielded important new information on the release of peptidergic and aminergic primary chemical messengers (hormones, transmitters and modulators). The mere presence of a peptide in a neurosecretory cell is clearly insufficient evidence of its function as a primary messenger. The demonstration of regulation of peptide synthesis, in a manner corresponding to its expected role, is proving a powerful tool in attributing specific functional roles to peptides in brain and endocrine tissues (LIGHTMAN). Knowledge of the amino acid sequence of a peptide precursor enables measurement and localization of messenger RNA in defined areas of highly complex tissues such as the nervous system. In this way, regional changes in secretory peptides can be assessed in response to appropriate stimuli. The application of molecular biology will increasingly permit detailed studies of the control of primary signalling molecules and the specific receptor molecules with which they interact. Nevertheless, more traditional experimental techniques, such as electron microscopy, continue to provide information on secretion. Novel preparative and staining methods (MORRIS and POW) are adding to our understanding of how membrane-bound secretory vesicles are mobilized and gain access to those regions of plasma membrane where exocytosis is observed. Ultrastructural studies have demonstrated dynamic interactions between neurosecretory terminals and adjacent glial cell processes (HATTON).

A central role for calcium in triggering secretion was established 20 years ago, largely based on studies of adrenal medullary (chromaffin) cells, the vertebrate motor end plate and invertebrate ganglionic synapses. Peter F. Baker, first at the Physiological Laboratory in Cambridge and subsequently at King's College, London, initiated detailed quantitative studies on the role of calcium in

stimulus–secretion coupling. His studies of calcium transport and distribution, together with his work on calcium channels, are major achievements. They provided a large part of the stimulus for the *Secretory Event* conference held at Kolimbari, Crete, in March 1988. Peter's untimely death has robbed this active field of experimental biology of its leading participant.

The contribution of calcium from both intracellular and extracellular sources to the control of secretion can now be assessed as a result of the introduction of calcium-sensitive dyes such as fura-2 (MASON *et al.*; LEONG), which, together with antibody localization of intracellular calcium-handling proteins, has led to a revision of the role of calcium in secretory cells. Intracellular calcium oscillations, the frequency and amplitude of which may encode intracellular signalling information, have been described (THORNER, HOLL and LEONG). For some tissues evidence has been obtained that specialized subcellular particles, 'calciosomes', are deployed specifically for calcium sequestration (POZZAN *et al.*). Electroporation, pioneered by Peter Baker and his colleagues, has provided a method for manipulating intracellular concentrations of free calcium, second messengers and a wide range of other probes (RINK and KNIGHT). A variety of methods all point to a central role in the secretory process of calcium released from intracellular stores. Another major advance has been the demonstration of guanine nucleotides (GILL, MULLANEY and GHOSH) and inositol-phosphate-activated calcium translocation mechanisms in secretory cells (GILL, MULLANEY and GHOSH). Progress has been made in investigations of the relationships between the formation and metabolism of inositol phosphates and calcium metabolism in the transduction mechanisms of signals received by secretory cells (PUTNEY). Distinct mechanisms for regulating secretory release by opioid receptors and β-adrenergic receptors have been identified in hormone-secreting nerve terminals (BICKNELL).

Several non-invasive optical methods have been introduced in an attempt to provide a correlate of the secretory event on an appropriate time scale. Light scattering has been used to probe the surface charge properties of granule and plasma membranes, leading to the conclusion that neutralization of membrane surface charge is not a calcium-specific step in granule discharge by exocytosis. Total intensity light-scattering changes have been detected on the millisecond time scale (SALZBERG and OBAID), and quasielastic light-scattering findings point to a breakdown of an intracellular matrix in the nerve terminal (SATTELLE) – a finding in accord with immunocytochemical evidence for disassembly of cytoskeletal elements as a central step that permits membrane–vesicle interactions (AUNIS and BADER). The identification of molecules, such as synexin, which promote membrane fusion during exocytosis is accelerating our knowledge of the molecular basis of granule–membrane interactions (POLLARD, BURNS and ROJAS).

Patch-clamp and voltage-clamp electrophysiology have been applied successfully to exposed neurosecretory nerve terminals and cultured secretory cells. In this way events that couple stimulus reception and secretory terminal activation

are under investigation (McBURNEY and KEHL). Capacitance changes in the secretory terminal membrane can be monitored with a precision that enables direct detection of exocytotic events (PENNER and NEHER). Measurement of this dynamic event, which has hitherto proved elusive, is a major advance. The possibility of applying patch-clamp and/or capacitance measurements whilst simultaneously monitoring changes in intracellular messenger molecules is a measure of how far this field has progressed in recent years and offers the prospect of a complete description of the secretory event in the near future. The collection of papers presented at the conference in Kolimbari is evidence of rapid progress towards this goal, and is dedicated to the memory of Professor Peter F. Baker FRS who did so much to pioneer and to advance research on stimulus–secretion coupling.

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