The endocrine versatility of the gut: general and evolutionary aspects of the active peptides of the gastrointestinal tract

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Recent years have seen an unprecedented expansion of interest in the gastrointestinal endocrine system that shows no signs of abating. In large measure this awakening can be attributed to the chemical studies that have resulted in the isolation and elucidation of structure of a wide variety of biologically active gut peptides (Gregory and Tracy, 1975; Mutt, 1976). The availability of highly purified preparations of these peptides has made possible detailed studies of their effects and mode of action at the cellular level. In addition, it has become possible to apply immunochemical methods of analysis that have helped to reveal the cellular origins of the peptides and have allowed their estimation in blood and tissue extracts. Several unexpected findings have emerged from these studies. For example, it now seems possible that some of the peptides produced by gut endocrine cells are not secreted into the blood stream, but rather act locally by diffusion to their targets through the extracellular space (paracrine effects). Furthermore, it is now clear that many of the active peptides in gut extracts originate not just in gut endocrine cells but also in nerve fibres. Peptides of the enteric plexuses are also found in the central nervous

system, and other peptides previously identified in brain have since been found in the gut. These developments raise questions of fundamental importance about the interrelationships of the brain-gut peptides and their roles in health and disease that, taken together, point to the need for a re-evaluation of the system of peptide messengers as a whole. An important aspect of such an analysis is the extent to which the chemical and functional relationships of these peptides can be accounted for in evolutionary terms. The relevance of this approach is emphasised by the similarity in structure of groups of brain-gut peptides that suggest a shared ancestry, both of the molecules in question, and of the entire system of neuronal and hormonal peptides.

Interrelationships of nerves and endocrine cells

At least eight peptides have been reported to occur in mammalian gut endocrine cells and in central or peripheral nerve fibres (Table 1). Only substance P and neurotensin have been isolated from both tissues (Leeman *et al.*, 1977); much of the evidence for the distribution of the other peptides rests on

Peptide	Gut nerves	Gut endocrine cells	Brain	Amphibian skin glands	Other tissues
Cholecystokinin	+	+	+	} Caerulein	(Phe-Met-Arg-Phe-NH ₂ in mollusc nerves)
Gastrin	+	+	+		
VIP	+	+	+		
Glucagon	—	+	—	—	Vertebrate pancreas
GIP	—	+	—	—	
Secretin	—	+	—	—	
Motilin	—	+	—	—	Protochordate gut endocrine cells Insect ganglia
Somatostatin	+	+	+	—	
TRF	?	?	+	+	
Bombesin	+	+	+	+	Eleudoisin in cephalopod salivary gland
Substance P	+	+	+	Physaelemin	
Neurotensin	—	+	+	Xenopsin	
Enkephalin	+	+	+	—	Vertebrate pituitary
Chymodinin	?	?	—	—	
ACTH	—	+	+	—	

Table 1 Distribution of peptides found in vertebrate gut extracts

+ Identified by isolation and/or immunochemical methods; — absent, or not yet reported; ? present in extracts but cellular origin uncertain.

radioimmunoassay or immunocytochemistry, and the possibility cannot be excluded that these substances exist as distinct but cross-reacting molecules in the brain and gut. As yet, cellular aspects of synthesis and secretion have been studied for only a few peptides, but the balance of evidence favours the view that both hormonal and neuronal peptides are synthesised initially as large precursors which are sequestered into secretory granules or vesicles, converted by proteolytic and other enzymes to yield the active peptide, and are then released by exocytosis or a related process. There may also be similarities in the electrical excitability of nerves and endocrine cells (Tischler *et al.*, 1977). The differences between neuronal and hormonal peptides can therefore be seen to lie largely in the mode of their delivery to target cells: on the one hand classical neurotransmitters diffuse across a synapse to act at a post-synaptic site, and on the other hand hormones are released into the blood stream and so transported to their targets. However, between these two extremes there are a variety of intermediate situations. For example, some nerves, notably those associated with the hypothalamus and neurohypophysis, secrete hormonal peptides directly into the blood stream. Other neuronal peptides are apparently not released either at synapses or capillaries, but may nevertheless mediate nerve-nerve interactions with slower onset and over longer periods than is generally associated with classical neurotransmitters (Barker, 1977). Similarly, it is thought that in the gut some endocrine cells might release peptides which diffuse to, and act on, adjacent mucosal cells in a local or paracrine mode of regulation (Pearse *et al.*, 1977). Thus, there is no straightforward distinction to be made between the mode of action of neuronal and hormonal peptides; instead there is an almost continuous spectrum between true hormones on the one hand and true neurotransmitters on the other.

The evolutionary origins of peptides that function as extracellular molecular messengers are still uncertain, and are in any case part of a larger problem that includes other aspects of cell-cell interaction, such as the origins of the regulation of growth and differentiation in multicellular organisms. There is evidence that nerves specialised for the secretion of peptides, so called neurosecretory neurones, are present in the most primitive of metazoans (the coelenterates) and so can be considered an ancient feature established early in the evolution of nervous systems (Scharer, 1978). The coelenterate neuropeptides are thought to control growth and development, and since there is no circulatory system in these animals they must presumably diffuse to their targets. Paracrine-like regulation is

thus one of the earliest forms of extracellular control to be mediated by peptides.

On present evidence, peptide-secreting glandular endocrine cells appear to be absent from the lower metazoans, so that, in a sense, peptidergic neurones can be considered ancestral to the endocrine cells of higher species. This need not imply direct evolutionary descent of endocrine cells from peptidergic neurones, although Pearse (1975) has presented the evidence for this case. The ability to produce and secrete active polypeptides occurs widely throughout both vertebrates and invertebrates. For example, substance P is present in mammalian nerves and gut enterochromaffin cells; in some amphibian species there are also high concentrations of substance P-like peptides (physalaemin) in skin, and a related peptide (eledoisin) has been isolated from the salivary gland of the cephalopod mollusc, *Eledone*. The wide distribution of substance P-like peptides is not unique, and other groups of peptides, such as those related to bradykinin, also exhibit a wide distribution. In mammals, bradykinin is generated in the peripheral circulation by the action of proteolytic enzymes on a precursor protein produced in the liver, but related peptides again occur in high concentrations in the skin of some amphibia, as well as in the venom secretion of certain wasps (Bertaccini, 1976). Both examples serve to illustrate the fact that related active peptides or their precursors can be found in a variety of quite distinct systems with no obvious phylogenetic or functional relationships. Conceivably, the related peptides of these diverse systems may have arisen by a process of convergent evolution. It seems more reasonable to suppose, however, that these and probably other peptides were established early in evolution and have since been strongly conserved. Obviously, active peptides form only one link in a chain of communication that necessarily includes other elements, such as appropriate target organ receptors and postreceptor transducing mechanisms. Once established, the capacity to employ a peptide as a molecular messenger might be drawn upon independently by systems as different as brain and gut by virtue of changes in the pattern of gene expression in these tissues. This is not particularly surprising for there are obvious advantages to be gained by deploying in more than one biological context a single system of extracellular communication molecules, for which the necessary genetic information is already available. Set in this light the dual distribution of peptides in gut and brain can be seen as an act of biological economy or conservation.

The dual function of a molecule as both hormone and neurotransmitter is not particularly novel, the

catecholamines being a well known case in point. The successful application of the same messenger in two different systems depends on the adequate separation of these systems. Segregation can be achieved both functionally and morphologically. Thus, the blood-brain barrier presents an obvious obstacle to the penetration of circulating peptides into the nervous system. Within the CNS the release of peptides at specific nerve endings must inevitably restrict their sites of action. In addition there might be differences in the requirements for concentrations of the same peptide at different targets such that only locally released peptide can achieve concentrations sufficient to evoke a response.

Phylogeny of the vertebrate brain-gut peptides

The dual distribution of peptides in nerves and gut endocrine cells was probably established early in the evolution of the vertebrates. The point is well illustrated by cholecystokinin-like peptides (Table 2). We have recently purified from sheep brain a peptide with the sequence of the C terminal octapeptide of porcine cholecystokinin (CCK 8), and we have also isolated a second octapeptide which is slightly less acidic but otherwise has identical biological and immunochemical properties to CCK 8 (Dockray *et al.*, 1978). CCK 8 has been identified immunochemically in extracts of hog intestine together with the cholecystokinins of 33 (CCK 33) and 39 residues isolated by Mutt (1976), and a component which is probably of intermediate size (Dockray, 1977a). In extracts of the brain and gut of the lamprey we have found factors which have immunochemical and chromatographic properties and biological actions resembling, although not identical to, those of CCK 8 (Holmquist *et al.*, 1979). Lampreys are of special phylogenetic interest since they are living representatives of the earliest of the vertebrate groups, the Agnatha, and have been separated from the rest of the vertebrate line

(Gnathostomata) by over 500 million years of evolution. The distribution and structure of CCK-like peptides have therefore changed relatively little throughout the course of vertebrate evolution, suggesting a strongly conserved biological role for these molecules in brain and gut. This conservation is even more striking when one considers the cellular origins of these peptides in the gastrointestinal tract. Recent morphological studies indicate that in the lamprey the gut endocrine cells that contain CCK-like factors resemble in ultrastructure and histochemical properties the gut endocrine cells of mammals (Van Noorden and Pearse, 1974). Similar cells have also been found in protochordates, like amphioxus (Van Noorden and Pearse, 1976). Thus the organisation of cells producing CCK-like peptides, and probably other gut peptides, appears remarkably constant throughout the vertebrates, in spite of profound evolutionary changes in their target organs, for example development of stomach and pancreas. It would seem then that these cells are uniquely suited to responding to the presence of food in the gut lumen by the secretion of peptides which regulate digestive activity.

Molecular evolution of active peptides

Insight into the evolutionary history of the mammalian gut hormones and related peptides can be obtained by consideration of their amino-acid sequences. The identical C terminal pentapeptide sequences in porcine gastrin and cholecystokinin suggests that the two hormones evolved from a common ancestor (Table 2). Likewise, the related sequences in glucagon, secretin, vasoactive intestinal polypeptide (VIP), gastric inhibitory polypeptide (GIP), and possibly bombesin and chymodinin suggests a shared history (Barrington and Dockray, 1976; Dockray, 1977b). The existence of these molecular families can be accounted for by the sequential operation of two distinct processes: in the first instance gene duplication must occur to produce two daughter genes each coding for the peptide; in the second instance point mutations in the structural gene lead to amino-acid substitution and hence divergence of the peptides. Only one of the daughter genes is required to fulfil the role of the original gene so the other is relatively free to diverge in structure. Not all amino-acid substitutions are of equal importance. Substitutions in the functionally important parts of the molecule will cause marked changes in biological activity, usually decreasing activity. Such mutations will therefore face strong selective pressure and are unlikely to survive. Consequently, amino-acid substitutions in the biologically important parts of a molecule will be less

	H or R
Porcine gastrin, antral mucosa	-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH ₂
Porcine/sheep CCK, brain and intestine	R -Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH ₂
Caerulein, amphibian skin gland	R Glp-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH ₂
Molluscan neuropeptide	Phe-Met-Arg-Phe-NH ₂
R = SO ₃ H	

Table 2 *Origins and structures of gastrin and cholecystokinins*

frequent than elsewhere, which explains why the conserved regions of gastrin and CCK include the minimum sequences essential for biological activity.

However, gene duplication and divergence are not the only types of mutation that can alter the structure of peptides. Track (1973) has proposed that secretin, glucagon, and gastrin may have arisen from a frameshift mutation in the insulin gene preceded by gene duplication. Frameshift mutations occur where there is a shift by one or two bases in the reading frame of DNA codon triplets. Such mutations are unlikely to have been responsible for the origins of many peptide hormones since they lead to the production of a completely new peptide that in the absence of appropriate target organ receptors will presumably be biologically inactive. Other possible types of mutation include those affecting splicing of mRNA at the post-transcriptional level. Recent work indicates that several structural genes (for example those for ovalbumin, B globin, etc) are composed of regions that are not translated interspersed between those that are. The regions that are not expressed are believed to be enzymically spliced from the mRNA before translation. Mutations in the splicing regions could lead to translation of different sequences of the gene either instead of, or in addition to, the usual sequence. Finally, there is good evidence that many secretory peptides are the product of post-translational processing of large precursor molecules that are the initial product of mRNA translation. Several types of processing enzymes are likely to be involved. Thus, proteolytic enzymes cleave the precursor peptide to produce smaller active peptides, while other enzymes are responsible for modifications such as the C terminal amidation and sulphation of tyrosine that occurs in gastrin and CCK. Mutation of the processing enzymes will inevitably lead to differences in the structure of the final peptide. Several pieces of indirect evidence support the idea of differences in processing pathways that might be the result of natural selection. For example, CCK 8 and CCK 33 have been isolated from brain or gut in the form of molecules possessing a sulphated tyrosine residue in the seventh position from the C terminus (Mutt, 1976; Dockray *et al.*, 1978), and unsulphated forms have not so far been identified. The sulphate group is known to be essential for the full biological potency of the peptide and there will therefore be strong selective pressure to maintain the efficiency of the sulphation system. In contrast, antral gastrin occurs in about equal amounts of sulphated and unsulphated forms, and sulphation is known to have little or no effect on the potency of the hormone in stimulating acid secretion. The enzyme system required for sulphation of gastrin is therefore under

less pressure than that for CCK, which might explain why it is less efficient.

There might also be differences in the processing pathways for a particular peptide in different tissues. For example, there has recently been controversy over the presence of VIP in nerves and endocrine cells. Early reports suggested that VIP-like immunoreactivity was present in endocrine cells in all parts of the gut (Polak *et al.*, 1974). More recently, several studies have failed to confirm this observation and have suggested, instead, that VIP is present in the nerve plexuses (Larsson *et al.*, 1976). The discrepancy might be explained by the presence in nerves and endocrine cells of different immunoreactive forms of VIP that varied in their cross reactivity with different antisera. In keeping with this idea, we recently showed that in extracts of human colonic mucosa, which contains both nerve fibres and endocrine cells, there were, in addition to a form similar to or compatible with the peptide originally purified from hog intestine, at least two other immunoreactive forms of VIP (Fig. 1). In contrast, in human colonic muscle, which contains nerves but not endocrine cells, there was the single form similar to the original porcine peptide (Dimaline and Dockray, 1978). On this evidence it seems that octacosapeptide VIP has a neuronal origin in the gut,

Fig. 1 Elution profile of acetic acid extract of human colonic mucosa (upper panel) and human colonic muscle (lower panel) after fractionation on CM-Sephadex. Immunoreactive VIP in the column eluates was estimated by radioimmunoassay using an antiserum specific for the NH_2 terminal region of porcine VIP. (Reproduced from Dimaline and Dockray, 1978.)

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while the molecular variants are present in endocrine cells. This observation could be explained by different processing pathways for VIP in nerves and in endocrine cells (Fig. 1).

Receptors

It is clear that during the course of evolution, molecules which are identical or closely related to the mammalian gut hormones have developed physiological roles not just in the control of digestion but also in the regulation of metabolism and, in the central nervous system, as neurotransmitters or neuromodulators. The evolution of these peptides cannot therefore be considered solely in terms of changes in their molecular structure and cells of origin, but must also take into account the evolution of their target cells. The importance of cell surface receptors in mediating the action of hormones and neurotransmitters is now well established. In particular, considerable progress has been made in the identification and characterisation of the receptors for some mammalian peptides. The results of these studies provide a basis for understanding in molecular terms the evolutionary relationships between peptides and their target cells.

Thus, in mammals, cell surface receptors binding secretin, VIP, and glucagon have been identified on liver cells and fat cells (Bataille *et al.*, 1974). On both cells there are receptors with high affinity for VIP and low affinity for secretin that do not bind glucagon; the receptors binding glucagon do not bind VIP or secretin. Thus, in addition to the divergence of glucagon, VIP, and secretin, there have also developed different receptors specific for these peptides. It is tempting to speculate that these might have arisen by an analogous process of duplication and divergence. In this context recent studies on the exocrine pancreas are particularly revealing. Gardner and co-workers (1978) have described a population of receptors on guinea pig pancreatic acinar cells that have high affinity for VIP and low affinity for secretin, while a second population of receptors have high affinity for secretin and low affinity for VIP. These results are based on experiments using dispersed pancreatic acinar cells *in vitro* and the possibility cannot yet be completely dismissed that the two types of receptor are present on different cell types. There are estimated to be about 135 000 receptors per cell with high affinity for secretin and low affinity for VIP and about 9000 per cell with high affinity for VIP and low affinity for secretin (Gardner *et al.*, 1978). In mammals such as dog and rat, VIP is a weak stimulant of pancreatic exocrine secretion, whereas secretin is a strong stimulant. However, we have found that in birds (turkey)

Fig. 2 Response of the pancreas in urethane anaesthetised turkeys to pure natural porcine secretin (●) and pure natural porcine VIP (○). Basal secretion is marked by X. Note that the range of doses of secretin was about 10 times higher than that of VIP. On a molar basis VIP is 25–30 times more potent than secretin in stimulating the flow of pancreatic juice. (Reproduced from *Experientia*, 29, 1510, 1973.)

porcine secretin is a weak stimulant of the flow of pancreatic juice (Fig. 2). In addition, the secretin-like peptide isolated from chicken intestine by Nilsson (1974) also has low potency on the avian pancreas (R. Dimaline and G. J. Dockray, unpublished observations). In sharp contrast, VIP (porcine or chicken) is a strong stimulant of the flow of pancreatic juice from the avian pancreas (Dockray, 1978). The threshold dose is about 10 ng/kg which is comparable to the dose of porcine secretin needed to stimulate the pancreas in mammals. Although the regulation of the bird exocrine pancreas is still poorly understood, the available data would be consistent with a role for VIP analogous to that of secretin in mammals. The different responses of the pancreas in birds and mammals could be explained by differences either in the relative numbers, or in the specificities, of pancreatic cell receptors for secretin and VIP. Confirmation of this is now needed by direct studies

of the avian pancreatic receptors. It is significant, however, that the affinity of mammalian hepatocytes for secretin is about 1% of that for VIP, whereas the affinity of chicken hepatocytes for secretin (porcine) is less than 0.1% of that for VIP (Bataille *et al.*, 1977). VIP and its liver receptors are presumably a common inheritance of birds and mammals from their reptilian ancestors, and since the separation of these two lines there would seem to have been evolutionary changes in the affinity of this receptor for secretin. In a general sense, these studies reveal the scope for variability of target organ receptors, both in terms of numbers of receptors per cell and specificity for different ligands. In the evolution of new target-hormone relationships, such as must have accompanied the development of the stomach and pancreas, the existence of a pool of variability in both peptides and receptors must have been of crucial importance.

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