Brain and gut peptides *

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The first evidence that the same peptides could be present in both the brain and the gut was given in 1931 with the discovery of substance P (Von Euler and Gaddum, 1931). In spite of this early start, the important revelation that there were a large number of peptides with this dual localisation and also with powerful and extensive pharmacological actions took more than 40 years to materialise. After the finding of large quantities of the hypothalamic peptide somatostatin (Arimura *et al.*, 1975) in the gastrointestinal tract, increasing numbers of peptides have been found to be common to the gut and brain. To date the list includes somatostatin, substance P, cholecystikinin (CCK), enkephalin, neurotensin, vasoactive intestinal polypeptide (VIP), and bombesin. A number of these peptides, such as somatostatin, neurotensin, and enkephalin, were originally found in the brain and later in the gut, whereas others, such as VIP, bombesin, and gastrin, were found first in the gut.

There is increasing evidence that outside the brain these peptides are localised in typical endocrine cells, or in fine efferent nerve fibres, or both, as part of the autonomic innervation. Although some peptides

may show a preferential localisation to either cells or nerves (Fig. 1) no clear-cut distinction can be seen between the peptides which may be localised to one or both of these structures. The classical division of the autonomic nervous system into adrenergic and cholinergic parts, originally proposed by Langley and Anderson (1895), was recently challenged by Baumgarten *et al.* (1970) who described another type of neurosecretory granule with an ultrastructure different from that of the adrenergic or cholinergic types. Baumgarten's granules were much denser and larger than the classical autonomic nervous system vesicles and were termed p-type for their resemblance to the central nervous system peptidergic neurosecretory granules responsible for the production of oxytocin and vasopressin.

It is now well accepted that among the neuronal peptides, VIP at least is present in this p-type of neurosecretory granule.

Technology

Information on the distribution, cellular localisation, and precise quantities of the peptides in a tissue sample can be given by two immunological procedures, radioimmunoassay (RIA) of tissue extracts and fluids to determine the quantities of peptides

Fig. 1 Diagram showing the 'spectrum' of localisation of neuropeptides to both cells and nerves of the gastrointestinal tract. At one end, substance P (Sub P), enkephalin (ENK), and vasoactive intestinal peptide (VIP) are mainly found in nerves, while at the other end somatostatin (SRIF), cholecystikinin (CCK), and neurotensin (NT) are mainly in cells. Bombesin (BN) appears equally in both structures.

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present, and immunocytochemistry (IC) of tissue sections to establish their location. These two techniques, especially when used in combination, have provided extremely valuable insights.

Two major problems are frequently encountered by immunocytochemists.

(1) The neuropeptides are water soluble and therefore easily washed out of a tissue section. It is thus extremely important to render the peptide insoluble without altering its antigenic site. This cannot be done by conventional fixation but is best achieved by the use of two cross-linking agents, diethylpyrocarbonate vapour (DEPC) (Pearse *et al.*, 1974), or p-benzoquinone, used either as a vapour or as an aqueous solution (Pearse and Polak, 1975).

(2) Specificity. Antibodies to peptide hormones

are raised to an insoluble complex composed of the pure peptide coupled covalently to a larger molecule, such as albumin. The resulting antiserum will contain a mixed proportion of desirable specific and unwanted non-specific antibodies. To ensure that only the specific antibodies participate, it is extremely important to dilute the antiserum to the maximal extent. This is possible with recently developed immunocytochemical procedures, which use high dilutions with long incubation times, particularly when combined with the peroxidase-antiperoxidase (PAP) staining procedure (Sternberger, 1974).

It is also extremely important to use a range of well-characterised antibodies as well as rigorous controls, the latter including the use of antiserum after absorption with the antigen being studied.

Fig. 2 Section of human gut (muscle wall) fixed in a solution of p-benzoquinone and stained with antibodies to (A) synthetic substance P, and (B) pure porcine VIP. Note the classical beaded appearance of the nerve fibres. (Original magnification $\times 252$.)

Individual peptides

SUBSTANCE P

The undecapeptide substance P was discovered incidentally by Von Euler and Gaddum (1931) while they were working on the distribution of acetylcholine in the gut. Substance P exerts powerful pharmacological actions mostly on smooth muscle and the vasculature (Powell *et al.*, 1978). It has been localised by immunocytochemistry mainly to fine nerve fibres of the gut wall (Fig. 2A). The production of substance P by ganglion cells in the gut was until recently controversial, but has now been fully substantiated by the use of tissue culture methods (Schultzberg *et al.*, 1978; Jessen *et al.*, 1979).

Substance P is also present in scattered endocrine, argentaffin cells (enterochromaffin or EC cells) of the gut mucosa which also simultaneously store serotonin. The largest concentrations of substance P in the central nervous system are found in the dorsal roots of the spinal cord, in the pons, medulla oblongata, hypothalamus, mesencephalus, and amygdaloid complex (Hökfelt *et al.*, 1978).

In addition, an important network of substance P-containing nerves has recently been found in many other organs such as the lung (Wharton *et al.*, 1979), genitourinary tract, and skin. The distribution of these nerve fibres, particularly beneath the epithelial layer of areas of high tactile sensitivity, suggests a sensory role for substance P.

Substance P has been found, by bioassay, to be absent from the aganglionic segment of the gut in Hirschsprung's disease (Tafari *et al.*, 1974) and Chagas's disease (Hial *et al.*, 1973). Large quantities of substance P have been found by RIA in the circulation of patients with gastrointestinal carcinoids

which are known to originate from EC cells (Powell *et al.*, 1978), and in extracts of such tumours.

SOMATOSTATIN

Somatostatin is a 14-amino-acid peptide which was originally extracted from the hypothalamus and identified as the factor which inhibits the release of growth hormone from the pituitary (Brazeau *et al.*, 1973). It was later found that somatostatin is present in large quantities in the gastrointestinal tract (Arimura *et al.*, 1975). It exerts powerful inhibitory actions on the release of a number of hormones from the pituitary and the gastrointestinal tract (see below).

ACTIONS OF SOMATOSTATIN

Inhibition of: growth hormone, thyroid stimulating hormone, insulin, glucagon, pancreatic polypeptide, gastrin, secretin, insulin-dependent insulinotropic hormone, motilin, enteroglucagon, gastric acid, gastric emptying, pancreatic bicarbonate, pancreatic enzymes, gall bladder contraction, myoelectric complexes, coeliac blood flow, xylose absorption.

In the gut, somatostatin is found mostly in the gastric antrum and the pancreas. Immunocytochemical methods show it to be localised to endocrine cells (Fig. 3). These have been characterised and recently internationally accepted, as the D cells of the ultrastructural classification (Polak *et al.*, 1975).

In the brain, somatostatin is found not only in the hypothalamus but also in many other areas including pons, medulla, amygdaloid complex, limbic area, and hippocampus (Hökfelt *et al.*, 1978). Ultrastructural and immunocytochemical studies have shown that brain somatostatin is present in neurosecretory granules of a larger size (170 nm diameter) than those storing acetylcholine and

Fig. 3 Somatostatin cells in human antrum. Note distribution of cells on basal membrane. (Original magnification $\times 203$.)

adrenaline (Pelletier *et al.*, 1977). Somatostatin has also been found in the thyroid gland (Van Noorden *et al.*, 1977), the thymus, and in sympathetic ganglia containing catecholamines (Hököfelt *et al.*, 1978).

In spite of the fact that recent reports indicate the presence of somatostatin in the pancreatic and gastric effluent blood (Harris *et al.*, 1978), its wide and powerful range of actions, as well as its distribution throughout the body, suggests that somatostatin must act mainly in the capacity of a paracrine (local) hormone. The term paracrine was originally introduced by Feyrter (1938) to indicate a 'local' action of the clear cells of his 'diffuse endocrine system'. Feyrter suggested that these clear cells acted upon neighbouring non-endocrine epithelial cells.

Numerous single or mixed somatostatin-containing endocrine tumours of the gut and pancreas have recently been described (Larsson *et al.*, 1976; Ganda *et al.*, 1977; Alumets *et al.*, 1978; Bloom *et al.*, 1978b).

The powerful inhibitory actions of somatostatin on the secretion of pancreatic and gastrointestinal hormones suggest that somatostatin may be one of the several factors whose excess or deficiency may play a part in the development of duodenal ulcer disease (Polak *et al.*, 1978d). Somatostatin deficiency may also be involved in many cases of severe intractable neonatal hypoglycaemia with hyperinsulinaemia. Samples of pancreas removed from sick children show a significant decrease of extractable and stainable somatostatin (Fig. 4) and alteration of the normal arrangement of the insulin/somatostatin-secreting cells (Polak *et al.*, 1978a).

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)
VIP was originally extracted from the porcine

duodenum and found to be a 28 amino-acid peptide closely related to glucagon, secretin, and glucose-dependent insulintropic peptide (GIP). It has been shown to exert powerful pharmacological actions on smooth muscle and blood vessels, as well as influencing the secretory activity of the gastrointestinal tract (Said, 1978). The role of VIP as a neurotransmitter or hormone is discussed elsewhere in the Symposium (page 63).

VIP is very widely distributed. It has been found to be present not only in the brain and gut but also in the genitourinary tract (Bishop *et al.*, 1979), pancreas (Polak *et al.*, 1978c), posterior pituitary (Van Noorden *et al.*, 1978), and salivary glands (Bloom *et al.*, 1978a). Immunocytochemical studies localise VIP mainly to fine nerve fibres of these organs. Using the technique of serial semithin (800 nm)/thin (80 nm) sections, with examination by immunocytochemistry and electron microscopy, respectively, has shown that VIP is stored in the p-type of neurosecretory granules originally described by Baumgarten (Polak and Bloom, 1978).

In the gut, fine VIP nerve fibres are found in the mucosa, the sub-mucosa, and the two muscle layers as well as in the plexi of Meissner and Auerbach (Fig. 2B). Cell bodies are also occasionally seen which contain VIP.

The involvement of VIP in the Verner-Morrison syndrome is discussed elsewhere (page 85).

An interesting recent finding is the remarkable increase of VIP nerve fibres in the affected gut segment in Crohn's disease (Polak *et al.*, 1978b). The VIP nerve fibres appear extremely hyperplastic, enlarged, and disorganised. There is twice as much extractable VIP, estimated by RIA, in the abnormal area of the gut as in comparable regions of normal

Fig. 4 (A) Normal pancreas (2-month-old term baby) showing the presence of numerous, well stained somatostatin cells. (B) Pancreas from a child with intractable hypoglycaemia showing scattered and poorly stained somatostatin cells. (Original magnification $\times 285$.)

gut wall. In contrast, another inflammatory bowel disease, ulcerative colitis, shows the normal number and distribution of VIP nerve fibres (Fig. 5A, B). A possible explanation may be that in Crohn's disease the inflammatory process, which affects all the gut layers, acts as a 'stimulus to proliferation' for the scattered intrinsic nerve cell bodies, whereas in ulcerative colitis the inflammation is only superficial and perhaps fails to 'irritate' the VIP cell bodies. It is possible therefore that estimation of gut VIP may become a useful tool for diagnosis in undetermined cases of inflammatory bowel disease.

ENKEPHALINS

The enkephalins are two closely related pentapeptides differing only by one amino-acid (methionine or leucine) at the C terminal end. They were first found in the brain, after the discovery of opiate receptors,

and during the search for a possible endogenous morphine.

One of them, met-enkephalin, belongs to a larger group of endogenous opiates, grouped under the generic name of endorphin (*endogenous morphine*). A larger peptide containing leu-enkephalin has not yet been found.

In the brain the enkephalins are found in areas related to pain transmission (Hökfelt *et al.*, 1978). Both met- and leu-enkephalins are found in the gastrointestinal tract (Polak *et al.*, 1977). The largest quantities are present in the gastric antrum and upper duodenum where they are localised to fine nerve fibres of the gut wall and occasional endocrine cells of the gut and pancreas. The cell bodies for these fine nerves are rarely seen. However, support for the location of enkephalinergic neurones in the bowel wall comes from the observation that enke-

Fig. 5 (A) Endoscopic biopsy of human colon from a patient with all the clinical and histopathological features of ulcerative colitis. Note the normal appearance of the VIP nerve fibres. (B) Section of human colon from a patient with Crohn's disease. VIP nerve fibres appear highly stained, thickened, and irregularly distributed. (Original magnification $\times 252$.)

phalin fibres can be demonstrated by immunohistochemical methods in cultures of Auerbach's plexus (Schultzberg *et al.*, 1978; Jessen *et al.*, 1979).

The localisation of enkephalins in the gastrointestinal tract may provide an explanation for the powerful effects of morphia, known from antiquity, on many functions of the gastrointestinal tract. Furthermore, it has recently been shown that enkephalins exert powerful modulating effects on the smooth muscle, blood flow, and secretions of the gut (Konturek, 1978).

BOMBESIN

Bombesin is a 14 amino-acid peptide originally extracted from amphibian skin by Erspamer and Melchiorri (1973). It was then shown to have many potent pharmacological actions in mammals on the gut, the brain, the lung, and the urinary tract.

The distribution of bombesin-like material has been analysed by combined RIA and immunocytochemistry. Large quantities of bombesin are found in the brain (hypothalamus, thalamus, limbic system, and amygdaloid complex), gut (throughout the entire length), and lung (fetal and neonatal). Chromatographic analysis of the bombesin-like material in mammals indicates the presence of two molecular forms, one which elutes from gel columns in the same position as amphibian bombesin and another larger form (Polak *et al.*, 1978e).

Immunocytochemical techniques localise bombesin to fine efferent nerve fibres of the gut wall and to endocrine cells of the bronchial mucosa, where they are found singly or in small groups (Wharton *et al.*, 1978). It has recently been shown that bombesin is a powerful, pH independent, gastrin releaser. It is thus possible that bombesin plays a key role in gastrointestinal disorders associated with abnormalities of acid secretion.

Neurotensin and cholecystokinin are discussed by Dr Blackburn (page 12) and Professor Rehfeld (page 26).

Conclusions

At the end of the 19th century the control of gut functions was considered to be mediated almost entirely by the nervous system. This concept formed the theory of 'nervism' postulated by Pavlov. The discovery, by Bayliss and Starling, of 'chemical messengers', or hormones, which have a powerful influence on gut function, caused the replacement of the nervism theory by the new concept of endocrine control.

The latest discovery of a peptidergic component of the autonomic nervous system shows that there is a

major area of overlap between the classically separated endocrine and nervous systems and reconciles the previously opposing views of Pavlov and Bayliss and Starling. It is now realised that the two control systems, far from being antagonistic, are highly synergistic and interdigitated. It also strongly supports Pearse's hypothesis (1969) of a common origin in the embryonic neuroectoderm of the peptide-producing endocrine cells and the neurones of this significant and powerful diffuse neuroendocrine system.

There are several unexplained effects of autonomic nerve stimulation which cannot be altered by cholinergic or adrenergic blockade. These include vasodilation of the salivary gland (Heidenhain, 1872), pancreatic bicarbonate release (Hickson, 1970), and numerous pulmonary reactions (Richardson and Béland, 1976). The mechanism behind these effects can now be explained by the existence of this additional component of the autonomic nervous system, from which peptides can be released after electrical stimulation.

Gastrointestinal function as a whole is, generally, equally ill-understood. It is quite likely that many normal gut functions are the culmination of a well orchestrated sequence of actions by the agonistic and antagonistic modulators of the endocrine, nervous, and neuroendocrine systems.

It is clear that the study of a single aspect of gut physiology will yield insufficient information to solve any problem. We will have to achieve an overall understanding of how the peptides act, where they are localised, and what factors cause their release. Intriguing details are already emerging which promise an exciting outlook for future research into this new and fascinating discipline of gut neuroendocrinology.

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