Gastrointestinal Hormones

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Chemistry, isolation and purification of gastrointestinal hormones

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The isolation in the early 1960s (Gregory & Tracy, 1975; Jorpes & Mutt, 1973) from porcine intestinal and gastric antral tissues of the classical gastrointestinal hormones gastrin, secretin and cholecystokinin, the latter having also the properties ascribed to pancreozymin, confirmed the long-suspected peptide nature of these substances. It then seemed—for a time—that the field of gastrointestinal-hormone research had been revealed in its main features and that future work in it would proceed in obvious directions such as the isolation of the above-mentioned hormones from various different species and the isolation of other assumedly known hormones, such as enterogastrene, infection, villikinin, enterocrinin and others (Greengard, 1948), which still were in the state of physiological principles, something that those isolated had also been for many decades.

Some of those expected developments did actually take place; others did not, but unexpected discoveries have convincingly shown that the field is far more complicated than previously believed and that much more work remains to be done before we will recognize all that is in it.

All gastrointestinal hormones are still peptides, but this is partly because of a somewhat arbitrary definition, since substances like prostaglandins and biogenic amines also may exert hormone-like actions on organs of the digestive tract.

Other definitions, such as a gastrointestinal hormone being biosynthesized in a stomach or of the gastrointestinal tract or hormone-like actions on organs of the digestive tract, have proved to be untenable (Pears, 1976). Entero-gastrene was transformed from a unique substance to a concept since several different intestinal peptides have been found to inhibit gastric acid secretion (Gregory, 1967; Johnson & Grossman, 1971). The possibility still remains, however, that some hitherto-unrecognized peptide, or other type of substance, having enterogastrone activity as its main physiological function may exist. Incretin has similarly converted from substance to concept (Creutzfeldt, 1979).

Several peptide hormones, such as insulin (Abel, 1926) and also glucagon (Staub et al., 1953), had been isolated by techniques that were developed at the very beginning of peptide-hormone chemistry, namely fractionations of aqueous hormone solutions with organic solvents or neutral salts, isoelectric precipitations of impurities or of the active substances, etc. Applied to gastrointestinal hormones, such techniques had resulted in varying degrees of purification but not in isolation. A retrospective explanation for this undoubtedly is that all gastrointestinal hormones have been found to occur in their tissues of origin in very low concentrations, much lower than that of insulin and glucagon in the pancreas. Isolation became possible when the older separatory techniques had been replaced by, or complemented with newer, more efficient, methodology. Techniques such as chromatography on cellulose-based ion-exchangers, counter-current distribution, and molecular sieving became available in the 1950s and made the progress of the 1960s possible. These methods have remained a mainstay in preparative work in the field until quite recently, when newer techniques, like high-performance liquid chromatography, affinity chromatography, isoelectric focusing, isochophoresis and others, seem to be turning a page and introducing a new era of accelerated progress.

Isolation of gastrointestinal hormones has been greatly facilitated by their relative thermostability, which has made it possible to remove both enzymes and structural tissue proteins at an early stage of the preparative procedures by heat denaturation. Two complementary extraction procedures have been in use for many years. One devised by Gregory & Tracy (1975) for gastrin entails extraction of the tissue with boiling water, followed by adsorption of the peptides from the extract to aminoethyl- or diethylaminoethyl-cellulose and elution with a weakly alkaline solution (Gregory, 1973). This technique works well for other acidic peptides also (Dockray et al., 1978), presumably because acidic peptides are not adsorbed to tissue proteins, most of which carry a negative charge at neutral pH values (Jorpes, 1968). It may be mentioned that already Edkins (1906) and later Blair et al. (1961) had observed that gastrin could be extracted by boiling water.

The other technique, worked out in our laboratory (Jorpes & Mutt, 1973), is adapted for basic and neutral peptides, and excludes the very acidic ones. The tissue is briefly boiled in water and then extracted with cold dilute acetic acid. The rationale being that extraction with cold acid should minimize cleavage of acid-labile peptide bonds. The initial boiling does not extract other than small amounts of secretin, as had been found already by Bayliss & Starling (1902). From the extract the peptides are adsorbed to alginic acid and eluted with 0.2 M HCl. It may be mentioned that the extraction method for substance P that finally led to its isolation from horse intestine (Studer et al., 1973) entailed boiling in only weakly acidified water (Pernow, 1953). Substance P had, however, then already been isolated from bovine hypophysial tissue by Chang & Leeman (1970) by using extraction with acid aqueous acetone, i.e. an extraction method similar to that used in work on insulin. Carraway & Leeman (1973) discovered neurotensin in bovine hypothalamic extracts prepared by the same type of extraction procedure they had used for substance P, and the identical peptide has, with the same technique, been isolated also from bovine intestine (Carraway et al., 1978).

Enough of extraction technology! Some 15 peptides have by now been isolated from intestinal and gastric tissues and many
more will certainly be isolated. Many of the latter will presumably turn out not to be hormones. Of those already isolated, however, all except the calcium-binding protein (Huang et al., 1975), and the intrinsic factor (Olesen et al., 1976) seem at least pharmacologically to exhibit hormonal properties.

From a physiological point of view, secretin, gastrin, cholecystokinin and the gastric inhibitory peptide, also called the glucose-dependent insulinotropic polypeptide (Brown & Pederson, 1976), seem definitely to have their hormonal status established (Grossman, 1977), whereas the situation in this respect is less clear for motilin (Brown & Dryburgh, 1978) and the vasoactive intestinal peptide (VIP) (Said, 1978) as well as for gastrointestinal substance P, neurotensin, somatostatin (Pradayrol et al., 1978), and two peptides, a porcine peptide with N-terminal histidine and C-terminal isoleucine amide (PHI) and another with N-terminal tyrosine and C-terminal tyrosine amide (PYT) recently isolated in our laboratory (K. Tatemoto, unpublished work). Peptide PHI has previously been referred to as ‘PI-HIA-27’ (Mutt, 1978). A peptide with bombesin-like pharmacological properties has recently been isolated from porcine non-antral gastric tissue, and in this case also its physiological role remains to be established (McDonald et al., 1979). The latter peptide is incidentally the first to be isolated from this section of the gastrointestinal tract, although the presence there of a peptide apparently identical with pancreatic glucagon has been known for 30 years (Sutherland & de Duve, 1948), and more recently the partial purification of it has been described (Doi et al., 1976). Enteroglucagon I, or glicentin, a peptide with both N- and C-terminally extended glucagon has been isolated from porcine small intestine (Moody et al., 1978).

Partial purification of peptides with glucagon-like immunoreactivity from porcine colonic tissue has been described (Conlon et al., 1979). Urogastrone (Gregory, 1975) has not been isolated from any gastrointestinal tissue, but material with urogastrone-like immunoreactivity has been shown to be present in the glands of Brunner (Elder et al., 1978).

Chemically all the gastrointestinal hormones and hormone candidates that have been isolated are single-chain peptides composed, as are other mammalian and many non-mammalian peptide hormones, of such L-α-amino acid residues as are ordinarily found in proteins. No glycopeptides have been found, but a glycoprotein from urine has been found to have enterogastone activity (Niada et al., 1979), and such activity has previously also been described for glycoprotein(s) found in human antacid gastric juice (Piasse et al., 1968). The only non-peptide constituent that has been found in gastrointestinal hormones is sulphuric acid, which esterifies the phenolic group of a tyrosine residue in cholecystokinin and may, or may not, esterify such a residue in gastrin. This sulphation has been found to have little influence on the potency with which gastrin stimulates gastric acid secretion in several mammalian species, but to strikingly increase it in the bullfrog (Grossman, 1970). For stimulation of gall-bladder contraction by cholecystokinin, the ester sulphate has something of the character of a co-hormone, the action of non-esterified cholecystokinin being very weak, although still noticeable at high hormone concentrations (Ondetti et al., 1970). For the pancreozyminic action of cholecystokinin, sulphation is also important, although less so than for the cholecystokinin action (Johnsen et al., 1970). Active C-terminal fragments may be obtained from cholecystokinin as has been found with gastrin (Gregory & Tracy, 1975). Bodanszky et al. (1978) found that replacement of the residue of tyrosine O-sulphate in the synthetic C-terminal heptapeptide of cholecystokinin by a residue of serine O-sulphate resulted in a drastic decrease in cholecystokinin activity. However, replacement by ε-hydroxyornleucine sulphate gave an analogue with fairly strong activity. It seems, therefore that the position of the acidic sulphate group in relation to the peptide backbone is the main determinant for its effect on cholecystokinin activity. Three disulphide bridges occur in urogastrone (Gregory, 1975) and one in somatostatin (Brazeau et al., 1973). A disulphide bridge has also been stated to occur in chymomodin, the complete amino acid sequence of which has, however, not yet been published (Adelson, 1975). Except for these three peptides, all other known gastrointestinal hormones and hormone candidates lack cysteine or cystine residues. It is now well known that peptide hormones may exhibit size heterogeneity due either to extracellular (Skeggs, 1956) or intracellular (Steiner et al., 1974) proteolytic modifications. Such heterogeneity has hitherto been described for gastrin and for cholecystokinin (Rehfeld & Amdrup, 1979; Noyes et al., 1979).

Cholecystokinin, gastrin, secretin, substance P and vaso-
active intestinal peptide, all have C-terminal amidic structures. Since there is evidence for such amidic bonds being formed by cleavage of peptide chains (Smyth, 1975; Suchanek & Kreil, 1977), there is reason to believe that, in all these cases, C-terminally extended peptides remain to be discovered. Indeed, for gastrin, a recent report indicates that such an extended gastrin may have been found (Gregory, 1979). N-terminal pyroglutamyl structures occur in gastrin (Gregory & Tracy, 1975) and in neurotensin (Carraway et al., 1978). Sequence similarities have been found both among gastrointestinal hormones themselves and among these and other peptides. It is evident from Table I that secretin, glucagon, vasoactive intestinal peptide, peptide PHI and gastrointestinal polypeptide may be grouped together on the basis of such similarities, whereas gastrin and cholecystokinin, together with the amphibian peptide caerulein (Erspamer et al., 1978) fall into another group, and the non-antral gastric heptadecapeptide, together with bombesin (Erspamer et al., 1978) into a third. It may be seen, however, that there is a certain similarity between the N-terminal sequences of cholecystokinin and the non-antral gastric heptadecapeptide, particularly if a one-residue deletion is assumed (McDonald et al., 1979). Porcine motilin, FVPIFYGELQRMQEQKERNKQG*

shows some sequence similarity to both gastrin and secretin (Mutt, 1978), equine (and bovine) substance P RPKQQFQFGLM
to xenopsin (Erspamer et al., 1978). The C-terminal tripeptide of substance P is, in addition, similar to that of secretin. The finding that, in one species, five different hormonal peptides clearly belong to one group raises the questions as to how many peptides belonging to the various groups still may remain to be isolated and what the biological significance of such variety is. Comparisons of the sequence similarities, and differences among the various hormonal peptides, have, when correlated with their biological activities, together with activity determinations on various synthetic analogues and derivatives of the peptides, shed some light on structural requirements for the different types of hormonal activities. For instance, gastrin and cholecystokinin have identical C-terminal pentapeptide amidic sequences, and both contain a residue of tyrosine O-sulphate. However, this residue is in gastrin linked directly to the pentapeptide, whereas in cholecystokinin it is displaced from the latter by one intervening amino acid residue. This displacement leads to greatly increased cholecystokinin potency in mammals (Ondetti et al., 1970; Johnson et al., 1970), although evidently not in the salmon (Vigna & Gorbman, 1977). In secretin the N-terminal histidine residue has been shown to be important, although not essential for hormonal activity (Solomon et al., 1977).

Although, then, a considerable amount of information is available concerning the amino acid residues that in varying degrees are important for the actions of the different gastrointestinal hormones, nothing definite is yet known about the mechanisms by which these hormones exert their actions. There is evidence that secretin increases the cyclic AMP concentration of pancreatic cells, but it is known neither how it initiates activation of the adenylyl cyclase nor how the increased cyclic AMP concentration is related to bicarbonate secretion (Case & Goebell, 1976). In contradistinction to secretin, cholecystokinin in its action on pancreatic acinar cells does not increase cyclic AMP concentrations but does increase cyclic GMP concentra-

* For a definition of symbols used in the one-letter notation for amino acid sequences, see Biochm. J. (1969) 113, 1-4. Q, pyroglutamine.
Evolutionary perspectives of peptides from gut endocrine cells and nerves

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The combined evidence of biochemical and histochemical studies indicates that the gut hormones are not an isolated system exclusively concerned with the regulation of digestion, but rather are part of a larger system of molecular messengers that includes putative peptide neurotransmitters, as well as other hormonal peptides (Dockray, Liverpool L69 3BX, U.K. [1978]). The combined evidence of biochemical and histochemical studies includes putative peptide neurotransmitters, as well as other hormonal peptides (Dockray, Liverpool L69 3BX, U.K. [1978]). Thus molecules identical or closely related to, gut hormones occur not only in endocrine cells of gut and pancreas, but also in central and peripheral nerves and in a variety of other tissues, including amphibian skin glands. The significance of this widespread distribution is still unclear, and a phylogenetic approach obviously offers an opportunity to gain further insight into this question, since the peptides common to nerves and endocrine cells are presumably related through a common evolutionary origin.

Structural relationships

Similarities in the amino acid sequences of gut hormones and other peptides suggests shared evolutionary histories (Dockray, Liverpool L69 3BX, U.K. [1978]). Thus molecules identical or closely related to, gut hormones occur not only in endocrine cells of gut and pancreas, but also in central and peripheral nerves and in a variety of other tissues, including amphibian skin glands. The significance of this widespread distribution is still unclear, and a phylogenetic approach obviously offers an opportunity to gain further insight into this question, since the peptides common to nerves and endocrine cells are presumably related through a common evolutionary origin.

(1) Gastrin and cholecystokinin. These peptides share a common C-terminal pentapeptide (Gly-Trp-Met-Asp-Phe-NH2). The amphibian skin-gland peptide, caerulein, also belongs to this group, since it has the same C-terminal octapeptide (CCK8 peptide) as pig cholecystokinin, except for a single amino acid substitution.

(2) Glucagon, secretin, vasoactive intestinal polypeptide and gastrin-inhibitory polypeptide. When these peptides are aligned from the N-terminus there are identical residues in two or more members of the group at all of the N-terminal 16 positions, and 5 of the next 11 positions.

(3) Substance P and bombesin. These two peptides share a common C-terminal dipeptide amide (Leu-Met-NH2). Bombesin was originally isolated from the skin of the amphibian Bombina bombina, but more recently a peptide closely resembling bombesin in its C-terminal portion has been isolated from pig stomach (McDonald et al., 1978).

(4) Insulin. In invertebrates, protostomes and the embryos of higher vertebrates, endocrine cells with insulin-like immunoactivity occur in intestinal mucosa, providing both phylogenetic and ontogenetic evidence linking this hormone with the gut endocrine system. Peptides related to insulin, or its precursors, proinsulin, have a widespread distribution, and include nerve-growth factor from salivary gland, relaxin from ovary, and the serum insulin-like growth factors. Natural selection acts to conserve functionally important residues. So, for example, it is no surprise that the conserved regions of gastrin and cholecystokinin include the minimal fragment (C-terminal tetrapeptide) of these two hormones with appreciable biological activity. Similarly, species differences in the sequences of gastrin and cholecystokinin occur in the middle and N-terminal regions, but, amongst mammalian species, are unknown in the C-terminal region. Some of the active peptides of the gut, e.g. motilin and somatostatin, are not related in sequence to the other peptides, and the evolutionary affinities of these molecules remain to be elucidated. Each of the main families of gut peptides contains at least one member that occurs in gut endocrine cells, and in one or both of central nerves; Some peptides occur in both endocrine cells and nerves. Peptides that are clearly related to members of these groups also occur widely in other tissues, e.g. the subretinal P-like peptides, phaeselein and eledoisin have been isolated from amphibian skin and cephalopod salivary glands respectively.

Nerve–endocrine cell relationships

The dual distribution of peptides in gut endocrine cells and nerves is very likely of great antiquity and was almost certainly established by the start of vertebrate evolution. Thus in lampreys, which are living representatives of the earliest vertebrate group (Agnatha), extracts of both brain and gut contain peptides with chromatographic, immunochemical and biological properties closely resembling those of mammalian CCK8 peptide (Holmquist et al., 1979). On present evidence there do not appear to be separate gastrins and cholecystokinins in the lampreys, indicating that cholecystokinin is the ancestral peptide for this group. The dual distribution of cholecystokinin-like peptides in brain and gut occurs throughout the vertebrate series and has obviously been strongly conserved (Dockray, 1979a). However, other peptides appear to have a variable representation in nerves and endocrine cells. The point is well illustrated by bombesin. By using antiserum raised to amphibian tetradecapeptide bombesin we have shown by radioimmunoassay and.