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Hormonal Polypeptides of the Upper Intestine

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When Bayliss and Starling discovered secretin in 1902 they did not call it a hormone. This word did not exist at the time, but was invented 2 years later as a consequence of the discovery of secretin (Bayliss, 1920). In the decades that followed its discovery numerous attempts were made to obtain secretin in chemically pure form. The early work has been the subject of several comprehensive reviews (Greengard, 1948; Grossman, 1950). Although non-toxic preparations that could be used in medicine were obtained, and also some of its chemical characteristics could be established, the isolation of secretin had to await the elaboration of more efficient techniques for the fractionation of polypeptide mixtures than were available to the early workers. When such techniques had become available further progress was possible and highly active preparations of secretin were obtained in several laboratories (Legge, Morieson, Rogers & Marginson 1957; Newton, Love, Heatly & Abraham, 1959; Fishman, 1959).

Pure (porcine) secretin was first obtained in our laboratory in 1961 (Jorpes & Mutt, 1961), and in the following year its (corrected) amino acid composition and partial structure were published (Jorpes, Mutt, Magnusson & Steele, 1962). The biological activity of the pure hormone is such that 1 μmol of secretin will induce the pancreas to secrete approx. 2.5mol of bicarbonate, in 0.1M solution.

The determination of its amino acid sequence posed no problems except that it had to be carried out on small quantities of polypeptide, since to obtain 1 μmol of secretin it was necessary to process the intestines of 3000 hogs. The amino acid sequence is: His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH_2 (Mutt, Magnusson, Jorpes & Dahl, 1965; Mutt, Jorpes & Magnusson, 1970). The sequence has been confirmed by synthesis (Bodanszky, Ondetti, Levine & Williams, 1967).

Secretin is strikingly similar to glucagon (Bromer, Sinn & Behrens, 1957). In 14 out of 27 positions the amino acids are identical in the two hormones, and in several other positions the differences are of a conservative nature. The difference between secretin and glucagon is no greater than may be found between polypeptides of identical function from distantly related species (Smith, 1967). Yet secretin and glucagon have widely different biological activities. In the method worked out for its isolation the destruction of secretin by the proteolytic enzymes of the intestine is prevented by heating the intestine for a few minutes in boiling water. This destroys the enzymes. From the coagulated proteinaceous mass secretin and other polypeptides are extracted with 0.5M-acetic acid, adsorbed from the extract on alginic acid, eluted with 0.2M-hydrochloric acid and precipitated from the eluate by saturating it with sodium chloride. The precipitate is a concentrate of the thermostable polypeptides of the intestinal wall, although not of all types.

The method of preparation obviously excludes very acidic peptides and also peptides of low molecular weight. From among the numerous peptides of this concentrate we next, after secretin, isolated a substance that exhibits the physiological properties ascribed to the hormone cholecystokinin (Ivy & Oldberg, 1928) and to the hormone pancreozymin (Harper & Raper, 1943), i.e. it both causes contraction of the gall bladder and leads to the secretion of enzymes from the pancreas (Jorpes, Mutt & Toczko, 1964; Jorpes & Mutt, 1966).

This substance is a polypeptide composed of 33 amino acid residues, the sequence of which has been determined (Mutt & Jorpes, 1968, and unpublished work). It is: Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr(SO_3)-Met-Gly-Trp-Met-Asp-Phe-NH_2.

The *C*-terminal pentapeptide sequence is identical with that of the gastrins (Gregory, Hardy, Jones, Kenner & Sheppard, 1964) and of caerulein (Anastasi, Erspamer & Endean, 1967). As in caerulein and in gastrin II there is also in cholecystokinin–pancreozymin a residue of tyrosine *O*-sulphate. In gastrin II this residue is linked directly to the *C*-terminal pentapeptide; in cholecystokinin–pancreozymin, as in caerulein, it is displaced from this by an interposed amino acid residue, threonine in caerulein, methionine in cholecystokinin–pancreozymin. Whereas no fragment with more than trace

activity has been obtained from secretin, the *C*-terminal octapeptide of cholecystokinin–pancreozymin shows a higher cholecystokinin and pancreozymin activity than the whole hormone, even on a molar basis (Ondetti, Rubin, Engel, Pluščec & Sheehan, 1970). In addition to the homology with the gastrins and with caerulein, cholecystokinin–pancreozymin shows some similarity to calcitonin (Bell *et al.* 1968; Potts, Niall, Keutmann, Brewer & Deftos, 1968). In the porcine hormones the amino acids are identical in positions 16, 20, 21 and 23. In position 17 there is an aspartyl residue in cholecystokinin–pancreozymin and an asparaginyl residue in calcitonin.

It has recently become evident that, in addition to secretin and cholecystokinin–pancreozymin, the wall of the upper intestine, and the polypeptide concentrates described above, contain several other biologically active polypeptides of probably hormonal nature. Two of these, one inhibiting gastric acid secretin (Brown, Mutt & Pederson, 1970), the other increasing blood flow to the splanchnic area, but also exhibiting several other activities (Said & Mutt, 1970), have been found to be chemically related to secretin and to glucagon (Said & Mutt, 1970; Brown, 1971).

Comparison of the amino acid sequences, when determined, of these four polypeptides, chemically related but with partly overlapping and partly different biological activities, may, it is hoped, shed some light on the structural requirements for these activities.

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Characteristics of Glucagon Action on the Hepatic Adenylate Cyclase System

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Hormone-sensitive adenylate cyclase systems are multimolecular communication devices integrated into the plasma membranes of target cells (Birnbaumer & Rodbell, 1969). In the rat hepatic system glucagon reacts specifically through non-covalent forces with a component, termed 'discriminator', that functions to regulate the activity of adenylate cyclase (Rodbell, Krans, Pohl & Birnbaumer, 1971*a*). Lipids play an essential role in the binding of glucagon and the regulatory function of the discriminator. Treatment of hepatic membranes with phospholipases and detergents results in concomitant losses of the binding and regulatory functions without affecting the activity and response of adenylate cyclases to fluoride ion, which acts directly on the enzyme (Birnbaumer, Pohl & Rodbell, 1971). Addition of phospholipids to treated membranes results in partial restoration of the binding and functional properties of the discriminator (Pohl, Krans, Kozyreff, Birnbaumer & Rodbell, 1971). The precise function of lipids in the binding process is unknown, but they may participate in the hydrophobic forces involved in binding of glucagon to the discriminator, as evidenced by the reversible inhibitory effects of low concentrations of urea and the marked sensitivity of binding to temperature (Rodbell, Krans, Pohl & Birnbaumer, 1971*b*).