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With 142 Figures and Numerous Schemes

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Gastrin and Cholecystokinin

An Arduous Task for the Peptide Chemist

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Summary

Both during synthesis and storage as well as handling of gastrin- and cholecystokinin-peptides serious difficulties related to a sequence-dependent high reactivity of various side chain functions were encountered. Thus, methods were devised to bypass or at least control side reactions along the synthetic routes, and analogs were designed to enhance the stability of the peptide factors with concomitant retainment of full hormonal potency. The access of these gastrin- and cholecystokinin-peptides allowed intensive physiological, biological and conformational studies aimed at a better understanding of the mechanism of action of this class of hormones. Additional attention was paid to particular derivatives well suited to improve the immunochemical methods as needed for a deeper insight into the physiological significance of the remarkable heterogeneity of these gut hormones.

Abbreviations: For amino acids and amino acid derivatives standard abbreviations recommended by the JUPAC-IUB commission on biochemical nomenclature were used.
Mox = methoxinine (oxa-analog of methionine); DCC = dicylohexylcarbodiimide; HONSu = N-hydroxysuccinimide; HOBt = 1-hydroxybenzotriazole; DMF = dimethylformamide; NMP = N-methylpyrrolidone; MEI = 2-morpholinoethylisocyanide; CCK = cholecystokinin-pancreozymin; HG = human gastrin, whereby the additional number indicates the chain-length of the gastrin peptide.
tlc = thin-layer chromatography; hplc = high performance liquid chromatography.

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The main structural characteristics of the gastrin-cholecystokinin family of peptide hormones are: 1) the common C-terminal tetrapeptide amide sequence Trp-Met-Asp-Phe-NH_2 which already in early structure-function studies on gastrin was recognized as the minimum fragment with physiological activity although at low potency, and thus as a kind of active center of the hormone (1); 2) the tyrosine-O-sulfate residue which for cholecystokinin (CCK) represents an essential structural feature for full hormonal expression, whereas for the gastrins its role has not yet been identified; they are isolated in the nonsulfated form I as well as in the sulfated form II (2,3) and no significant differences in their physiological actions have been detected so far; 3) the complex size-heterogeneity as resulting from the posttranslation processing of their precursor mole-

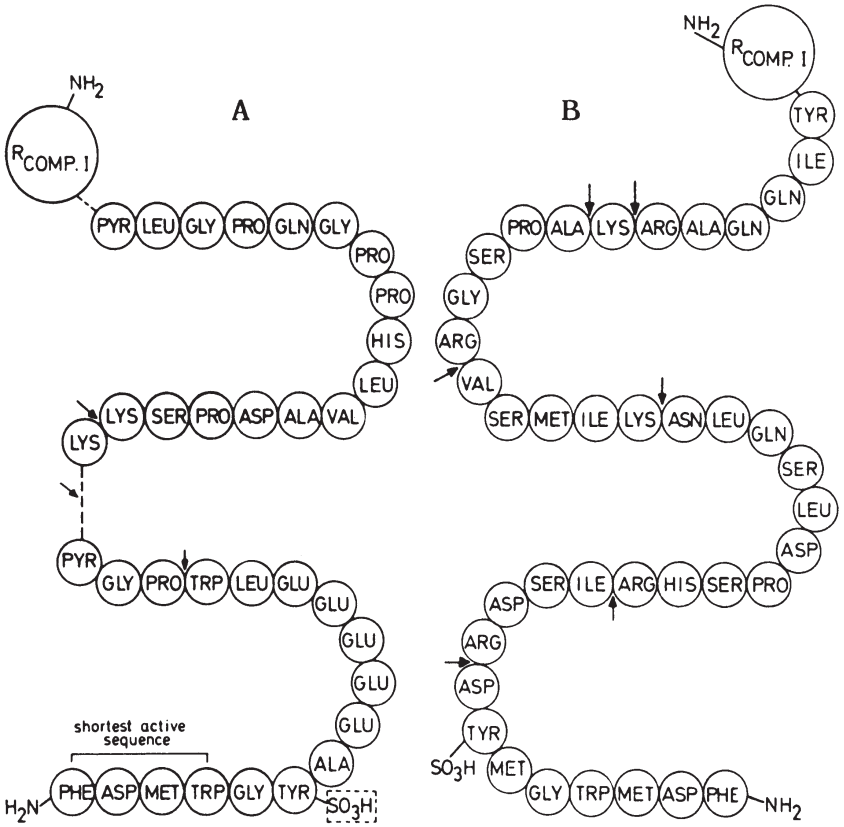

Fig. 1. Primary structures of A) human gastrins (HG) and B) porcine cholecystokinins. Arrows indicate enzymatic cleavages of the prohormonal molecules with production of gastrins and cholecystokinins of decreasing chain-length. The C-terminal amides result from enzymatic processing of glycine-extended forms

cules (Fig. 1) (4). To better understand the structural bases for the biological actions of this class of hormones and to possibly identify the physiological significance of the observed heterogeneity both in respect to sulfation and extension at the N-termini, extensive synthetic studies have been performed in the last years in our laboratory (5 - 15). Aim of these intensive efforts was i) to obtain the various components of this class of hormones at the highest possible degree of purity for structure-function studies ii) to identify fully active analogs stable on storage and handling as needed for physiological and clinical studies, and finally iii) to synthetize derivatives well suited for an efficient improvement of the immunological methods necessary for a reliable quantitative and selective evaluation of the different components both when circulating in the plasma and in the tissue of their biosynthesis. Our contributions in the field are reviewed in the following:

Synthesis of Gastrins

The main difficulties encountered in the synthesis of gastrin and CCK related peptides derive from those structural elements which are essential for the biological activity and thus, directly involved in the mechanism of action of this class of hormones, i.e. from the thioether function of methionine, the indole side-chain function of tryptophan and the tyrosine-O-sulfate moiety. This sequence-dependent enhanced reactivity particularly of the methionine and tryptophan residue towards oxidants and electrophiles as well as the instability of the sulfate-ester function has represented for years a challenge for the peptide chemist.

Oxidation of methionine: The pronounced tendency of the methionine residues in the gastrins and CCK-peptides to oxidation to the corresponding S-oxide derivatives with concomitant loss of activity (16 - 17) implies careful avoidance of traces of oxidants, e.g. peroxides and heavy metals along the various steps of synthesis and purification as well as of air-oxygen to prevent desactivation. Even if reduction of S-oxides with thiols is possible (18), handling of these peptides for in vitro and in vivo assays becomes delicate.

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