Different Synthetic Approaches to Luteinizing Hormone-Releasing Hormone (LH-RH)

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Several synthetic approaches to luteinizing hormone-releasing hormone (LH-RH), a decapeptidamide with the sequence pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 were evaluated.

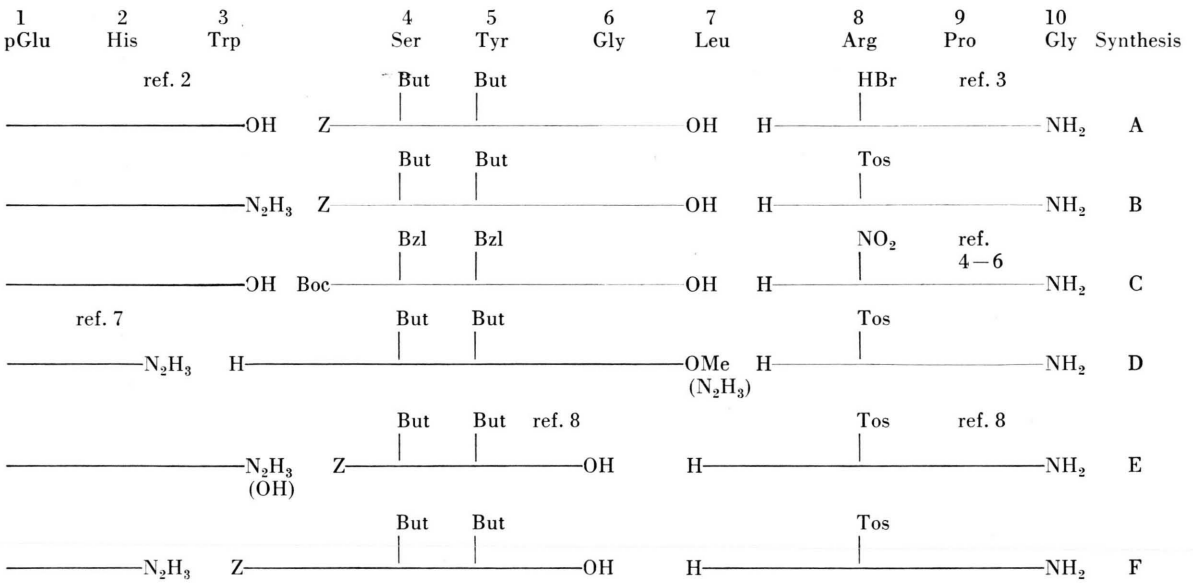
A solid phase synthesis was carried out according to the general procedure of Merrifield using dicyclohexylcarbodiimide (DCC) as coupling agent and the *tert*-butyloxycarbonyl (Boc) group for protection of the amino group of all amino acids except pyroglutamic acid. The latter was introduced in form of its pentachlorophenylester. For protection of the functional side-chains the benzyl group was used for serine and tyrosine, the dinitrophenyl group for histidine and the nitro group for arginine. The protected decapeptide was cleaved from the resin by transesterification. Subsequent treatment with HF, followed by ammonolysis and purification yielded pure LH-RH. When the peptide was directly cleaved from the resin by ammonolysis, considerable formation of ornithine instead of arginine containing material was observed, thus making the purification to homogeneous product extremely difficult [1].

The general strategy of the various synthesis by classical methods consisted in condensation of three preformed fragments by the azide, the DCC/hydroxysuccinimide (1-hydroxy-benzotriazole) or mixed anhydride procedure and is outlined in the scheme. These building blocks differed from each other either by variation of the protecting groups or by representing different sections of the decapeptide.

All protected decapeptides were deblocked by treatment with liquid HF, except the material obtained in synthesis A, where trifluoroacetic acid was used. Purification was achieved either by partition chromatography or by gel filtration and ion exchange chromatography. The final products of all syntheses were identical with each other, homogeneous, showing after chymotryptic digestion the expected three peptide fragments and possessed full biological activity.

As most convenient synthesis in our hands proved to be approach E. The C-terminal tetrapeptide was prepared by two consecutive mixed anhydride reactions of Z-Pro-OH with free glycine and of the resulting Z-Pro-Gly-OH [9] with ammonia, followed by hydrogenation and condensation with Z-Arg(Tos)-OH by the DCC procedure. This tripeptide was without further purification decarbobenzoxylated and coupled with Z-Leu-ONp to yield the cristalline protected tetrapeptide.

Fragments used in the different classical synhtheses [a].



[a] Standard abbreviations are used for amino acid residues and protecting groups. pGlu, pyroglutamic acid; Z, benzyloxycarbonyl; But, *tert*-butyl; Tos, Tosyl; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; OMe, OCH_3.

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Control of Secretion of Hypothalamic Hormones

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Data will be presented which indicate that the median eminence region of the hypothalamus is not directly responsible for the synthesis of the gonadotrophin releasing factors. It will be shown that the paraventricular region is specifically involved in the synthesis of FSH-RF, while the suprachiasmatic and the arcuate-ventromedial zones are specifically devoted to the synthesis of LH-RF.

Three experimental conditions will be discussed in which the intrahypothalamic stores of one gonadotrophin releasing factor have been modified without changing those of the other. These observations are not compatible with the hypothesis that one single hypothalamic factor controls the release of both LH and FSH as recently suggested.

In a series of *in vitro* experiments it has been shown that acetylcholine is able to liberate FSH from the anterior pituitary only if fragments of the basal part of the hypothalamus are present in the incubation media. These data have been taken as indicating that acetylcholine stimulates the release of FSH-RF from the incubated hypothalami, and that FSH-RF released under the influence of acetylcholine in turn enhances the secretion of FSH from the incubated pituitaries. It will be suggested that acetylcholine may play a major role in transferring extrahypothalamic influences to the neurons which synthesize the gonadotrophin releasing factors.

The synthetic decapeptide synthesized by Schally and his co-workers is able to release LH and FSH when injected into the carotid artery of the rat. The kinetics of the release of the two hormones under the influence of the decapeptide are quite different, LH being released more promptly than FSH. The activity of the decapeptide may be modulated by changing the levels of sex steroids in the general circulation. Apparently estrogens facilitate the release of LH, while androgens enhance the release of FSH.

Modulating Effects of Prostaglandins on the Release of Hypothalamic Hormones

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The prostaglandins are discussed as mediators of biological events in the hypothalamo-hypophyseal system. This phenomenon was first shown with respect to ACTH-release. A direct effect of PGE_1 was demonstrated by implantation into the medial basal hypothalamus. The effect of TRH on release of TSH *in vitro* from rat hemipituitaries is influenced by PGE_1. PGE_1 stimulates GH-release from bovine anterior pituitary tissue, PGE_1 and PGE_2 increase incorporation of labelled leucine into GH and prolactin. Zor *et al.* have studied cyclic AMP levels in anterior pituitary tissue following incubation with several prostaglandins, in order of potency being $PGE_1 > A_1 > B_1 > F_{1\alpha}$. None of them released LH in the system. Caldwell *et al.* have observed LH release by PGE_2 in a pituitary superfusion system. Both PGE_2 and $F_{2\alpha}$ stimulate LH release. Harms *et al.* have shown a neurotransmitter-like effect of PGE_2 after injection into the third ventricle PGE_2 increases plasma LH, while PGE_1 elevates prolactin. The stimulatory effects of prostagladins are antagonized or blocked by inhibitors of prostaglandin synthesis, *e. g.* 7-oxa-13-prostynoic acid. The *in vitro* effect of LH-RH on gonadotrophin release is modulated by prostaglandins. The evaluation of prostaglandin effects on hormone release