Short Communications

N-Terminal Amino Acid Sequence of the α -Subunit of Human Pituitary Luteinizing Hormone

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It is now well established that the pituitary glycoprotein hormones LH* (Papkoff & Samy, 1967), follicle-stimulating hormone (Papkoff & Ekblad, 1970) and TSH (Liao & Pierce, 1970), as well as the placental hormone chorionic gonadotrophin (Swaminathan & Bahl, 1970), consist of two subunits designated α and β (Pierce *et al.*, 1971a). Each hormone can be reversibly dissociated by treatment with reagents that do not rupture covalent bonds, the isolated subunits being of low biological activity. In each case partial or complete restoration of activity occurs on incubation of equal weights of α - and β -subunits in neutral aqueous solution (Papkoff & Samy, 1967; Papkoff & Ekblad, 1970; Liao & Pierce, 1970; Swaminathan & Bahl, 1970). Amino acid sequences have been reported for the subunits of sheep LH (Liu *et al.*, 1970, 1971; Papkoff *et al.*, 1971) and ox TSH (Liao & Pierce, 1971), and it is noteworthy that the sequences of sheep LH α -subunit and ox TSH α -subunit are nearly identical. These results, in conjunction with studies involving hybrid formation of the two subunits (Liao & Pierce, 1970; Pierce *et al.*, 1971b), implicate the β -subunits of LH, TSH and chorionic gonadotrophin as the determinants of hormone specificity, the α -subunits of each hormone being interchangeable. We now report the results of determination of the *N*-terminal 35 residues of human LH α -subunit and comparison of this sequence with published data on sheep LH α -subunit.

Human pituitary glands provided by the Medical Research Council were used for preparation of human LH (Hartree, 1966). Chromatography on CM-cellulose at pH 5.0 was used for final purification of LH and also for isolation of the α -subunit after dissociation of LH with 8M-urea (Nureddin & Hartree, 1971; Hartree *et al.*, 1971). For each series of sequence determination approx. 10mg of the reduced and carboxymethylated derivative of LH α -subunit was subjected to the automatic Edman degradation (Edman & Begg, 1967) with a Beckman automatic Sequenator and a Quadrol double-

*Abbreviations: LH, luteinizing hormone; TSH, thyroid-stimulating hormone (thyrotrophin).

cleavage program. The phenylthiohydantoin derivatives were identified by the combination of g.l.c., as described in the Beckman Sequencer Manual, and t.l.c. on plastic-backed fluorescence-impregnated silica-gel plates (Eastman no. 6060). Xylene-methanol (16:3, v/v), xylene-propan-2-ol (7:2, v/v) and butyl acetate-formamide-propionic acid-water (160:6:3:3, by vol.) were used as developing solvents, and identification of spots was aided by characteristic colours developed with ninhydrin. The phenylthiohydantoin derivatives of arginine and histidine were identified by the phenanthroquinone method of Yamada & Itano (1966) and by the Pauly reagent (Easley, 1955) respectively.

The amino acid sequence of the *N*-terminal portion of human LH α -subunit was investigated by Edman degradation. The series of sequential degradations was performed twice and the same result was obtained for each residue on each occasion. Our results are presented in Table 1, along with the sequence of the corresponding region of sheep LH α -subunit (Liu *et al.*, 1971; Papkoff *et al.*, 1971). Homology between the two sequences is apparent when human LH α -subunit *N*-terminal valine residue is aligned with residue no. 8 of the sheep LH α -subunit. This suggests that the *N*-terminal heptapeptide of sheep LH α -subunit is not essential for biological activity. Approx. 70% of the residues of human LH α -subunit are identical with those of the sheep LH α -subunit, and many others can be accounted for in terms of mutations involving a single base change. The five half-cystine residues have been conserved in both species, but notably the four lysine residues in this portion of sheep LH α -subunit have been replaced by neutral residues in the human LH α -subunit. We conclude that, although the cystine residues may have an important structural or functional role, the basic lysine residues in this portion of sheep LH may not be essential for activity. The identical sequence of 12 residues (nos. 24–35 in human α -subunit) in both species suggests that this portion of the polypeptide chain has an important biological function, perhaps in the interaction between subunits or with receptors in the target organ. This same sequence of 12 amino