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Long-lasting Corticotropic Action of [1-Gly]-ACTH-(1-18)-octadecapeptide Amide-poly-L-aspartic Acid Complex

The synthetic peptide [1-Gly]-ACTH-(1-18)-octadecapeptide amide (Gly^1-α^{1-18}-ACTH amide)[1] resembles in structure the first 18-amino acid sequence of natural ACTH, and, like the native hormone, has the activity of stimulating the production of adrenal corticosteroids in experimental animals.[2] Current studies on human subjects by Takebe, *et al.*[3] have also confirmed that it has a corticotropic activity comparable to that of natural ACTH. Since Gly^1-α^{1-18}-ACTH amide is chemically pure and a much smaller peptide than ACTH, and therefore less likely to give rise to allergic reaction as caused by impurities in the ACTH preparations extracted from natural sources or by antibody formation by the peptide itself, clinical application of the synthetic peptide would seem promising. However, therapeutic use of the peptide has so far been limited, as has as that of other ACTH derivatives, by its short biological half-life upon injection.[4] By combination of natural ACTH with gelatine, zinc hydroxide, carboxymethyl cellulose, or polyphloretin phosphate, several long-acting preparations have been obtained.[5] In these the prolongation has been attained either by delaying the flow into circulating blood or by protecting the peptide from enzymic inactivation at the extravascular injection site. Taking a hint from the water-insoluble complex described by Silman & Katchalski,[6] we have prepared a complex of Gly^1-α^{1-18}-ACTH amide with poly-L-aspartic acid (mol. wt. 2300) and have found that the complex has a significantly long-acting corticotropic activity upon intra-muscular injection in hypophysectomized rats. Similar results were obtained by forming complexes with poly-L-glutamic acid (mol. wt 5400), copoly-L-tyrosyl-L-glutamic acid (1:1, mol. wt. 21850) or poly-ethylene maleic acid (mol. wt. 9090). On the other hand, neutral or basic polymers such as poly-L-tyrosine or poly-L-lysine did not form complexes with Gly^1-α^{1-18}-ACTH amide and hence the activity was not prolonged. An acidic dipeptide alanyl-L-glutamic acid did not precipitate the hormone at any concentration tested. The poly-L-aspartate-Gly^1-α^{1-18}-ACTH amide complex was prepared as follows: 4 mg of poly-L-aspartic acid (Miles, AS 33A) was dissolved in 1 ml water containing a small amount of N NaOH to adjust the pH at 7.0, and the solution was combined with 1 ml of an aqueous solution of 4 mg of Gly^1-α^{1-18}-ACTH amide. A flocculent precipitate formed and the turbid solution was diluted with 2 ml of 40 mM Na_2HPO_4–KH_2PO_4 (pH 7) containing 1.8% of NaCl and 2% of benzyl alcohol. Five microliter aliquots of the suspension thus obtained were injected into the thigh muscles of Wistar strain male rats weighing 110 to 130 g 2 hr after hypophysectomy, and blood samples were collected at intervals from the abdominal aorta. The concentration of 11-hydroxycorticosterone (11-OHCS) in the plasma was determined by florimetry.[7] As reference, 4 mg of Gly^1-α^{1-18}-ACTH amide was dissolved in 4 ml of 20 mM phosphate buffer (20 mM Na_2HPO_4–KH_2PO_4, pH 7, with 0.9% of NaCl and 1% of benzyl alcohol), and 5 μl of this solution was intra-muscularly injected. As shown in Fig. 1, poly-L-aspartate-Gly^1-α^{1-18}-ACTH amide complex exerted a pronounced depot-effect persisting over a period of 6 hr, whereas the effect of an equivalent

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amount of Gly^1-α^{1-18}-ACTH amide was no longer detectable 2 hr after injection, although the maximum corticoid level was approximately the same. In a separate experiment, the effect of pretreatment with poly-L-aspartic acid on corticosteroidogenesis by Gly^1-α^{1-18}-ACTH amide was examined. Hypophysectomized rats were given 5 μl of Gly^1-α^{1-18}-ACTH amide (5 μg) in 20 mM phosphate buffer as described above, but 10 μl (500 μg) of poly-L-aspartic acid was pre-injected into a different site of the thigh muscle 10 or 60 min before the hormone was administered. The plasma 11-OHCS levels, 30 min, 1 and 2 hr after Gly^1-α^{1-18}-ACTH amide injection, were found to be essentially identical with those in animals not pretreated, suggesting that complex formation is necessary to cause the depot-effect. Amino acids analysis showed that the ratio of polymer to ACTH in the complex described above was 1: 2. On the other hand, the ratio was 1: 4, if a polymer of molecular weight 4460 was used. The former polymer consisted of 20 aspartic acid residues whereas the latter consisted of 39 aspartic acids. It thus seems probable that the carboxyl groups of poly-L-aspartic acid interact chiefly with the amino groups of the 8—18 portion of Gly^1-α^{1-18}-ACTH amide to form a complex which, after injection, dissociates gradually at a local site releasing ACTH into body fluid with a favourable rate to maintain a high plasma 11-OHCS level over a long period. Recent studies on the immunogenicity of polypeptide indicate that charged homopolymers such as poly-L-glutamic acid are usually less antigenic than hydrophobic polymers.[8] The immunological properties of ACTH-polymer complexes are under investigation.

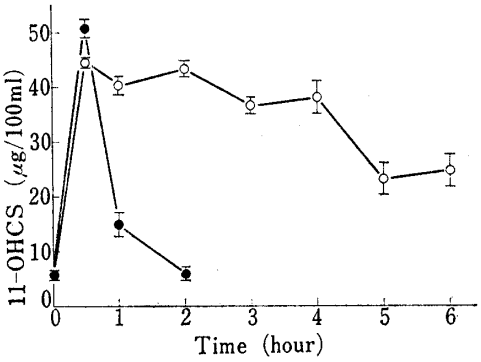

Fig. 1. Time Course of Plasma 11-OHCS Level in Hypophysectomized Rat Following Intra-muscular Injection of Gly^1-α^{1-18}-ACTH Amide (●——●) and Poly-L-aspartate-Gly^1-α^{1-18}-ACTH Amide Complex (○——○). Means ± S.E.

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Application of Dicyclohexyl Carbodiimide for Detection of Carboxylic Acids

Iron (III) complex of hydroxamic acid has long been used for the detection and determination of carboxylic acid derivatives. This reaction is simple and straightforward for carboxylic acid anhydrides, chlorides and esters.[1] However, the application to free carboxylic acids is cumbersome, because they should be first derivatized either to acid chlorides or esters. Furthermore, it is impractical to apply this reaction for the detection of carboxylic acids on paper or thin-layer.

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