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HUMAN GROWTH HORMONE ACTIVE SITE FOR MEMBRANE COOPERATIVE ENZYMES

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SUMMARY: The action of the human growth hormone (hGH) on the Hill coefficient for the inhibition by F^- of rat erythrocyte membrane acetylcholinesterase and for the inhibition by Na^+ of *Escherichia coli* membrane (Ca^{2+})ATPase was studied. The hGH and several synthetic fragments from hGH were able to decrease the n values in both systems. It can be concluded that 33 residues region in hGH, corresponding to positions 88 through 120, contains the "active site" for this hormonal action on membrane cooperative enzymes.

There is much justification for permanent research on the possible existence of a common active core(s) in pituitary growth hormones. This stems from the unfortunate fact that human subjects, suffering from hipopituitarism, do not respond to the structurally quite similar and more readily available growth hormones from other species. The concept that the active principle of human growth hormone (hGH) may be a small fragment of this protein is under continuous experimentation and debate in the field (1).

The use of membrane-bound cooperative enzymes as probes to record changes in the membrane due to the action of various hormones has been extensively illustrated (2-7). Also, as showed

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in the case of thyroid hormones, membrane cooperative enzymes are a highly specific tool to study the action of hormone analogues (7).

In this paper we show that hGH is able to change the cooperative behaviour of the membrane-bound enzymes: rat erythrocyte acetylcholinesterase and *Escherichia coli* (Ca^{2+})-ATPase. The combination of the assays thus developed with the availability of several synthetic fragments of hGH made possible to partially define the sequence responsible for this biological action. Human Growth Hormone is formed by a single polypeptide chain of 191 amino acids with two disulfide bridges (7). Part of the present work was reported in preliminary form (9).

MATERIALS AND METHODS

Rat erythrocyte acetylcholinesterase.

Male Sprague - Dawley rats, grown after weaning on basic diet supplemented with 5 % corn oil were used to obtain erythrocyte membrane-bound acetylcholinesterase. Details concerning erythrocyte preparations, assay of the enzymatic activities and calculation of kinetic parameters for the inhibition by F^- have been given in a previous article (6).

Escherichia coli (Ca^{2+}) *ATPase.*

The bacterial strain used was *E.coli* K-12 Hfr M_1 (pho^- alkaline phosphatase). The bacteria were grown aerobically in a gyratory shaker at 20°C in nutrient broth medium (3). Details of preparation of the membranes as well as those related to the procedure for the enzymatic assays and calculation of the kinetic parameters for the inhibition by Na^+ were previously described (10).

Human Growth Hormone

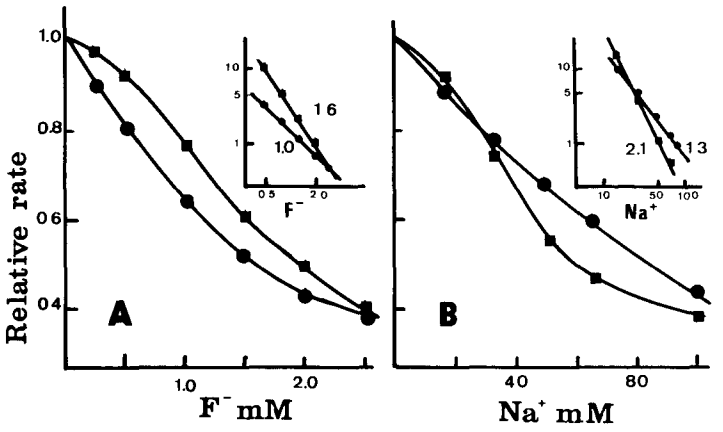
It was prepared according to Roos et al (11) with an additional step of purification by gel filtration.

Human Growth Hormone fragments.

They were synthesized by the solid phase method, as indicated before (12). All synthetic peptides had glycinamide as C-terminal amino acid and those containing a cysteine residue had the free sulfydryl blocked by an acetamidomethyl group. The synthetic peptides purified by gel filtration and partition chromatography on silica gel were characterized as single components by SDS gel electrophoresis, thin layer chromatography, amino acid composition and amino terminal residue detection*.

Human Growth Hormone and synthetic fragments were dissolved in water with the addition of small volumes of NaOH 0.1 M. Thereafter, the stock solutions were prepared in 310 mosM sodium phosphate (pH 8.0) or 20 mM HCl Tris buffer (pH 9.0) and were stored at -20°C until used.

* C.Peña, J.M.Stewart and A.C.Paladini (1978) manuscript in preparation.



ig.1.- Effect of the hGH on the Hill coefficient for the inhibition by F^- of rat erythrocyte acetylcholinesterase (A) and for the inhibition by Na^+ of *E.coli* (Ca^{2+})-ATPase (B). In the absence (■—■) and in the presence (●—●) of hGH 10^{-8}M. Inset shows Hill plots of same data. The same membrane preparation was used for the control and hormone tests.

TABLE 1: Effect of hGH fragments on the Hill coefficient.

hGH fragments added (0.18 µg/ml)	n values*	
	Acetylcholinesterase (rat erythrocyte)	(Ca^{2+}) ATPase (*E.coli*)
None	1.55 ± 0.05^a	2.20 ± 0.05^a
- 128	0.80 ± 0.10^b	---
19 - 128	0.90 ± 0.15^b	---
34 - 128	1.00 ± 0.10^b	---
44 - 128	1.05 ± 0.05^b	---
64 - 128	0.93 ± 0.08^b	---
88 - 128	0.90 ± 0.10^b	1.40 ± 0.10^b
98 - 128	1.55 ± 0.10^a	2.20 ± 0.15^a
108 - 128	1.53 ± 0.07^a	2.20 ± 0.10^a
81 - 120	0.90 ± 0.15^b	1.40 ± 0.02^b

* Mean of n values obtained with 3-5 different enzymatic preparations ± SEM. Values followed by different letters were significantly different ($p < 0.001$).

TABLE 2: Blocking action of the peptides hGH 98-128 and hGH 108-128 on hGH action.

Hormone or fragment added (M)	n values*	
	(Ca^{2+})ATPase (*E. coli*)	Acetylcholinesterase (rat erythrocyte)
None	2.20 ± 0.10^{a}	1.60 ± 0.05^{a}
hGH (8×10^{-9})	1.30 ± 0.10^{b}	---
hGH (8×10^{-9}) + hGH 98-128 (8×10^{-8})	2.20 ± 0.05^{a}	---
hGH (8×10^{-9}) + hGH 108-128 (8×10^{-8})	2.00 ± 0.05^{a}	---
hGH (8×10^{-9}) + hGH 108-128 (8×10^{-9})	1.50 ± 0.05^{b}	---
hGH 81-120 (8×10^{-8})	1.30 ± 0.03^{b}	1.00 ± 0.05^{b}
hGH 81-120 (8×10^{-8}) + hGH 98-128 (8×10^{-8})	2.20 ± 0.10^{a}	1.60 ± 0.05^{a}

* Mean of values obtained with 3-5 different enzymatic preparations $\pm$ SEM. Values followed by different letters were significantly different ($p < 0.001$).

TABLE 3: Specificity of the blocking action of peptides hGH 98-128 and hGH 108-128.

Hormone of hGH synthetic fragment added (M)	n values*
	(Ca^{2+}) ATPase (*E.coli*)
None	2.20 ± 0.05^a
Insulin (1×10^{-9})	1.40 ± 0.05^b
Insulin (1×10^{-9})+ 108-128 (8×10^{-7})	1.40 ± 0.05^b
L-T_3 (1×10^{-9})	1.00 ± 0.10^b
L-T_3 (1×10^{-9})+ 98-128 (8×10^{-7})	1.10 ± 0.10^b
hCS (1×10^{-8})	1.30 ± 0.05^b
hCS (1×10^{-8})+ 108-128 (8×10^{-7})	1.10 ± 0.15^b
hCS (1×10^{-8})+ 98-128 (8×10^{-7})	1.20 ± 0.10^b

* Mean of n values obtained with 3-5 different enzymatic preparations ± SEM. Values followed by different letters were significantly different ($p < 0.001$).

RESULTS AND DISCUSSION

Fig. 1 shows the action of hGH on membrane cooperative enzymes from both mammalian and bacterial systems. In the absence of the inhibitors (F^- or Na^+) the presence of hGH did not affect the specific activity of both enzymes. Several synthetic fragments from hGH were also able to decrease the Hill coefficient in the erythrocyte and *E.coli* systems (Table 1). The Peptides comprising the sequence of hGH from amino acid 1 through 128 as well as shorter ones like 19-128, 34-128, 64-128, 88-128, were as active as intact hGH. When the N-terminal amino acid was advanced past position 88 in the hGH polypeptide chain, the activity dissapeared (Table 1, peptides 98-128 and 108-128). Advancing the C-terminal residue from position 128 to position 120 did not affect the activity (Table 1, peptdie 81-120).

From those results it can be concluded that the 33-residues region in hGH, corresponding to positions 88 through 120, contains the "active site" for this particular action. Some closely related GH fragments, like hGH 95-136 and bovine GH 96-133 have been found

active in growth assays (13-14). Up to now though, these findings have not been generally confirmed (15).

The inative peptides 98-128 and 108-128 were able to block specifically the hGH action and that of the active peptide 81-120 (Table 2). These facts suggest the existence in hGH of only one "active site" responsible for its effect on membrane cooperative enzymes.

Table 3 shows that the inactive peptides 98-128 and 108-128 do not inhibit the action of hGH related (human chorionic somatomammotrophin, hCS) or unrelated (insulin, L-thriiodothyronine hormones. A high molecular specificity is thus suggested, in the blocking action of the inactive peptides.

We think that the introduction of this new methodological system in the hGH field may be important to detect the "in vivo" active core of the hormone.

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