PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 285-287 (1976)

Human Pituitary Growth Hormone. 44. Effects of Plasmin-Modified Hormone and Its Fragments on Ornithine Decarboxylase Activity and Lipolysis[1] (39193)

A. JAGANNADHA RAO, J. RAMACHANDRAN, AND CHOH HAO LI

Hormone Research Laboratory, University of California, San Francisco, California 94143

It has recently been demonstrated that HGH[2] retains its biological activity after limited digestion by human plasmin (1-3) with the removal of a hexapeptide fragment (3, 4) corresponding to positions 135-140 in the amino acid sequence of the HGH molecule (5). Reduction and alkylation of PL-HGH produced two peptide fragments (3, 4): one corresponding to amino acid residues 1-134 [Cys (Cam)^{53}-HGH-(1-134)] and the other to residues 141-191 [Cys (Cam)$^{165, 182, 189}$-HGH-(141-191)]. The NH_2-terminal fragment was found to retain approximately 14% of the biological activity as assayed by the rat tibia test and 6% potency in the pigeon crop-sac test (3), and was found to possess metabolic activities of HGH (4), whereas the COOH-terminal fragment had measurable growth-promoting and prolactin activities (3). Both fragments were also found to possess immunological activity as revealed by radioimmunoassay and complement fixation (6). Here we report the biological potency of PL-HGH and its fragments when tested *in vivo* for stimulation of hepatic ornithine decarboxylase activity in rats, as well as *in vitro* for lipolytic activity in isolated rabbit adipocytes.

Materials and methods. Human growth hormone was isolated as described by Li *et al.* (7) Plasmin-modified HGH and the two fragments were prepared according to the procedure of Li and Gráf (3). DL-[^{14}C]ornithine monohydrochloride (7.6 mCi/mmole) was obtained from New England Nuclear Corp.

Male rats, 21 days of age, of the Sprague-Dawley strain were injected ip with either 0.2 ml of the hormone preparation or saline. Four hours later, the animals were sacrificed, and the livers were removed and homogenized at 4° in 25 m*M* Tris-HCl buffer of pH 7.5 containing 0.1 m*M* disodium EDTA and 5 m*M* dithiothreitol. The homogenate was centrifuged at 20,000*g* for 60 min at 4°, and an aliquot of the supernatant was assayed for ornithine decarboxylase by the procedure of Jänne and Williams-Ashman (8).

Lipolytic activity was measured in fat cells isolated from the perirenal and epididymal fat pads of New Zealand White rabbits according to previously published procedures (9, 10). Protein estimation was done according to the method of Lowry *et al.* (11).

Results. Stimulation of hepatic ornithine decarboxylase activity in 21-day-old male rats by HGH, plasmin-treated HGH, and the two fragments are summarized in Table I. HGH produced a significant stimulation at a dose of 0.14 nmole and the response was proportional to the dose of the hormone. Plasmin-treated HGH appears to be more potent than HGH. The NH_2-terminal fragment was found to possess 10% potency of the native hormone. The COOH-terminal fragment was inactive at the highest dose tested (120 nmole).

The lipolytic activities of HGH, plasmin-treated HGH, and the two fragments are shown in Table II. Plasmin treatment seems to cause a drastic loss of lipolytic activity. Both plasmin-treated HGH and the fragments give a poor dose-response relationship; however, high doses of these derivatives cause significant lipolytic activity.

Discussion. Earlier studies with bovine growth hormone have shown that the hormone stimulates ornithine decarboxylase activity in the liver (12, 13). Our data in Table I confirm these observations with the human hormone. It is of interest to note that plasmin-modified HGH appears to be

[1] This work was supported in part by U. S. Public Health Service Grant No. AM-6097 and the Allen-Geffen Fund.

[2] Abbreviations: HGH, human growth hormone; PL-HGH, plasmin-modified human growth hormone.

TABLE I. STIMULATION OF HEPATIC ORNITHINE DECARBOXYLASE ACTIVITY BY HGH AND ITS DERIVATIVES.

Preparation	Dose (nmole)	Ornithine decarboxylase activity[a]	*P* values[b]
Saline	0.0	81.2 ± 8.4 (27)	
HGH	0.14	100.8 ± 14.8 (9)	<0.05
	0.42	161.2 ± 26.7 (19)	<0.005
	1.26	274.5 ± 11.0 (10)	<0.001
PL-HGH[c]	0.14	137.0 ± 11.0 (5)	<0.001
	0.42	274.0 ± 44.0 (5)	<0.001
Cys (Cam)^{53}-HGH-(1-134)[d]	3.10	109.7 ± 10.2 (10)	<0.05
	9.30	233.5 ± 45.2 (10)	<0.005
Cys (Cam)^{165,182,189}-HGH-(141-191)	40.0	97.7 ± 24.6 (5)	NS
	120.0	82.0 ± 32.0 (5)	NS

[a] Values (picomole $^{14}CO_2$ per milligram protein per hour) are the means ± SE. The number of animals is given in parentheses.
[b] Comparisons with basal values; NS, not significant.
[c] Relative potency to HGH, 130% with confidence limit of 34–1300 and $\lambda = 0.45$.
[d] Relative potency to HGH, 10% with confidence limit of 5–41 and $\lambda = 0.33$.

TABLE II. LIPOLYTIC ACTIVITY OF HGH AND ITS DERIVATIVES.

Preparation	Concentration (μM)	Glycerol production[a]	*P* values[b]
Saline	0.0	0.61 ± 0.08	
HGH	0.5	1.1 ± 0.03	<0.01
	1.5	2.7 ± 0.1	<0.01
PL-HGH	1.5	0.78 ± 0.08	NS
	4.5	0.96 ± 0.06	<0.05
Cys (Cam)^{53}-HGH-(1-134)	1.5	0.84 ± 0.1	NS
	4.5	0.97 ± 0.0	<0.03
Cys (Cam)^{165,182,189}-HGH-(141-191)	1.5	0.82 ± 0.08	NS
	4.5	1.17 ± 0.2	<0.01

[a] The values (μmole per gram dry weight cells per hour) are the means ± SE of triplicate determinations.
[b] Comparisons with basal values; NS, not significant.

slightly more active than the parent molecule. In this connection, it may be noted that Chrambach *et al.* (2) reported the enhancement of the prolactin activity of HGH by plasmin treatment.

Previous studies (3) showed that full growth-promoting activity is retained by plasmin-modified HGH and that the NH_2-terminal fragment also retained approximately 14% of this activity. The results presented here (Table I) indicate that the structural requirements for stimulating hepatic ornithine decarboxylase activity are identical to those observed for tibia growth. Thus, plasmin-modified HGH is fully active and the NH_2-terminal fragment about 10% as active as intact HGH. The NH_2-terminal fragment stimulated hepatic ornithine decarboxylase activity to the same extent as HGH, but higher doses of the fragment were required. The COOH-terminal fragment was devoid of the activity. These results suggest that the information necessary for the stimulation of ornithine decarboxylase activity is contained in the NH_2-terminal 134 amino acid residues of growth hormone. The amino acid residues from 141–191 may serve to protect the hormone from various degradative influences. Furthermore, both the NH_2-terminal and the COOH-terminal fragments were less soluble than the native HGH or the plasmin-treated HGH. The COOH-terminal fragment, which was least soluble, probably interacts with hydrophobic regions of the NH_2-terminal fragment to produce the stable globular structure of native HGH. Thus, even though the COOH-terminal fragment may have no direct role in the biological activity of HGH, it is required for optimal expression of the biological activity of the hormone.

Holladay *et al.* (14) reported that a cyanogen bromide fragment of ovine growth hormone consisting of two peptides corresponding to residues 6–124 and 150–179 of the molecule (15) linked by a disulfide bridge stimulated hepatic and renal ornithine decarboxylase activity but failed to show growth-promoting activity as measured by somatomedin induction.

The great loss of lipolytic activity following plasmin treatment of HGH (Table II) clearly suggests that the structural requirements for this biological property are differ-

ent from those required for skeletal growth and stimulation of ornithine decarboxylase activity. It also appears that the structural requirements for full lipolytic activity in isolated rabbit adipocytes are rigid, requiring the complete structure of the hormone.

Summary. The rat hepatic ornithine decarboxylase stimulating activity of plasmin-modified human growth hormone and its two peptide fragments has been investigated. The activity was completely retained after plasmin treatment. The NH_2-terminal fragment [Cys (Cam)^{53}-HGH-(1–134)] retained 10% of the activity, whereas the COOH-terminal fragment [Cys (Cam)$^{165, 182, 189}$-(141–191)] was not active.

The lipolytic activity of human growth hormone was greatly reduced after plasmin treatment, as examined in isolated rabbit adipocytes. It is suggested that the structural requirements for the lipolytic activity of the hormone are different from those required for stimulation of ornithine decarboxylase activity.

1. Reagan, C. R., Jr., Kostyo, J. L., Mills, J. B., and Wilhelmi, A. E., Fed. Proc. **32,** Abstr. 294 (1973).
2. Chrambach, A., Yadley, R. A., Ben-David, M., and Rodbard, D., Endocrinology **93,** 848 (1973).
3. Li, C. H. and Gráf, L., Proc. Nat. Acad. Sci. USA **71,** 1197 (1974).
4. Reagan, C. R., Jr., Mills, J. B., Kostyo, J. L., and Wilhelmi, A. E. Endocrinology **96,** 639 (1975).
5. Li, C. H., Proc. Amer. Phil. Soc. **116,** 365 (1972).
6. Clarke, W. C., Hayashida, T., and Li, C. H., Arch. Biochem. Biophys. **164,** 571 (1974).
7. Li, C. H., Liu, W. K., and Dixon, J. S., Arch. Biochem. Biophys. Suppl. **1,** 327 (1962).
8. Jänne, J. and Williams-Ashman, H. G., J. Biol. Chem. **246,** 1725 (1971).
9. Ramachandran, J., Lee, V., and Li, C. H., Biochem. Biophys. Res. Commun. **48,** 274 (1972).
10. Lee, V., Ramachandran, J., and Li, C. H., Arch. Biochem. Biophys. **169,** 669 (1975).
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
12. Jänne, J., and Raina, A., Biochim. Biophys. Acta **174,** 769 (1969).
13. Russell, D. H., Snyder, S. H., and Medina, V. J., Endocrinology **86,** 1414 (1970).
14. Holladay, L. A., Levine, J. H., Nicholson, W. E., Orth, D. N., Salmon, W. D., Jr., and Puett, D., Biochim. Biophys. Acta **381,** 47 (1975).
15. Li, C. H., Gordon, D., and Knorr, J., Arch. Biochem. Biophys. **156,** 493 (1973).

Received July 25, 1975. P.S.E.B.M. 1976, Vol. 151.