Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 256, No. 17, Issue of September 10, pp. 8882-8884, 1981
Printed in U.S.A.

Growth Hormone Stimulates Drug-induced Porphyrin Formation in Liver Cells Maintained in a Serum-free Medium*

(Received for publication, April 6, 1981, and in revised form, June 1, 1981)

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Human growth hormone and a few other related polypeptide hormones enhanced porphyrin formation induced by 2-allyl-2-isopropylacetamide in chick embryo liver cells maintained in a serum-free medium. The stimulatory effect of human growth hormone on drug-induced porphyrin formation was significant at 10 ng/ml and increased in a dose-dependent manner. Human growth hormone did not stimulate porphyrin formation in otherwise untreated cultures. These findings thus demonstrate a potentiating effect of growth hormones on hepatic porphyrin formation.

A number of hormones have been shown to stimulate heme synthesis in the liver (1-9). This stimulation is accomplished principally by the induction of δ-aminolevulinic acid synthase, the rate-limiting enzyme of heme biosynthesis in the liver (1). It has been difficult to demonstrate *in vivo* that changes in heme formation are a result of the actions of specific hormones. We have therefore developed a technique for culturing chick embryo liver cells in a completely defined medium free from components such as serum (1). Using this serum-free culture technique, it is also possible to show that insulin, cortisol, and triiodothyronine exert a permissive effect on the induction of δ-aminolevulinic acid synthase and porphyrin formation caused by 2-allyl-2-isopropylacetamide, a potent chemical inducer (1), or by a variety of natural steroid metabolites that have similar inducing properties (2).

Recently considerable evidence has accumulated to suggest that growth hormones may influence metabolic functions in many tissues (10). The observed effects of hormone administration *in vivo*, however, may not be due to its direct actions on target tissues, in that the hormone may act through a mediator produced in other tissues. To circumvent these problems we examined in the present study the influence of growth hormone and a number of related peptides on porphyrin formation in isolated chick embryo liver cells cultured in a serum-free medium.

* This work was supported in part by Grants ES-01055 and CA-15688 from the Public Health Service, by National Institutes of Health Grant AM-18677, and by Grant I-350 from the National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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MATERIALS AND METHODS

Highly purified human growth hormone (11), human choriomammosomatotropin (12), ovine prolactin (13), bovine growth hormone (14), plasmin-modified HGH[1] (15), HGH fragment (Cys(Cam)^{53}-HGH-(1-134)) (15), and reduced and alkylated HGH (16) were prepared as previously reported.

Cell cultures were prepared from 17-day-old chick embryos as described previously (1). Five hundred μl of cell suspension (1 volume of cell pellet in 200 volumes of a modified F12 medium (1) supplemented with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and, when appropriate, with growth hormone or other hormone preparations) were placed into a well of a Costar 3524 tissue culture "cluster-24" dish and incubated in a humidified atmosphere of 5% CO_2 and 95% air. The hormones were dissolved in 0.1 N NaOH, followed by dilution in a modified F12, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and pH of the hormone preparation was adjusted to 7.4 before addition to culture medium. After 24 h of incubation of cultures, the medium was replaced by 1 ml of fresh medium containing the same supplementation and an addition of AIA (100 μg/ml) was made. Incubations were carried out for a further 24 h. Porphyrins were extracted with 500 μl of 1 N perchloric acid:methanol (1:1, v/v) and determined spectrofluorometrically in a Hitachi-Perkin-Elmer MPF IV fluorescence spectrophotometer equipped with a red light-sensitive photomultiplier R928 as described previously (1).

RESULTS AND DISCUSSION

HGH, human choriomammosomatotropin, ovine prolactin, bovine GH, plasmin-modified HGH, a fragment of HGH (Cys(Cam)^{53}-HGH-(1-134)), and reduced and alkylated HGH alone showed no stimulatory effect on porphyrin formation at concentrations ranging from 10 to 5000 ng/ml (data not shown). In contrast, HGH enhanced the induction of porphyrin formation by AIA in a dose-dependent manner (Fig. 1). A significant effect was detectable at a hormone concentration of 10 ng/ml. An approximately 2-fold stimulation of porphyrin formation was observed with HGH at 100 ng/ml (Fig. 1). Porphyrin formation from added δ-aminolevulinic acid, however, was not affected by HGH in these cells (data not shown). The HGH fragment showed substantially less stimulatory activity on AIA-induced porphyrin formation as compared to the whole HGH, but the fragment was active at all concentrations tested (1 ~ 1000 ng/ml) as compared with the control cultures treated with AIA alone (Fig. 1).

Bovine GH and ovine prolactin were inactive at 100 ng/ml, but stimulated AIA-induced porphyrin formation approximately 2-fold at 1000 ng/ml, whereas human choriomammosomatotropin was inactive at all concentrations tested (Table I). The greater potency of HGH than bovine GH on a molar basis may be due in part to the monomeric nature of HGH in solution as opposed to the dimeric nature of bovine GH, and also to the greater solubility of HGH (17). The effect of plasmin-modified HGH on AIA-induced porphyrin formation appeared to be slightly greater than that of HGH (Table I). Reduced and alkylated HGH showed less activity than HGH, but at 1000 ng/ml it did stimulate AIA-induced porphyrin

[1] The abbreviations used are: HGH, human growth hormone; AIA, 2-allyl-2-isopropylacetamide; GH, growth hormone; HCS, human choriomammosomatotropin.

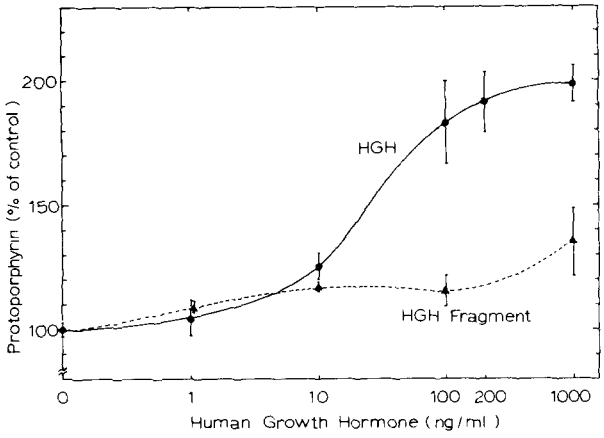

FIG. 1. **Effect of HGH on AIA-induced porphyrin formation in cultured chick embryo liver cells.** Cells were incubated in a modified F12 medium supplemented with each specific hormone as described. The medium was replaced with fresh medium containing AIA (100 μg/ml) after a 24-h incubation and cultures were further incubated for 24 h. Effect of hormone treatment on porphyrin formation is expressed as percentage of control cultures treated with AIA alone. Data are the mean value ± S.E. for 4 determinations.

formation by approximately 50% (Table I).

In serum-free cultures of chick embryo liver cells, insulin (1, 2, 18), cortisol (1, 2, 18), triiodothyronine (1, 2, 18), and cyclic AMP (19) were previously found to exert a permissive effect on drug-induced porphyrin formation. We therefore also examined the effect of HGH (100 ng/ml) in combination with these other permissive hormones in order to determine whether HGH may alter their effects. We found that HGH potentiated the permissive effects of cortisol (50 ng/ml), dibutyryl cyclic AMP (2 mM), and 8-bromocyclic AMP (2 mM), but had no significant potentiating effect when added to cultures with either insulin or triiodothyronine (Table II).

The results of this study provide for the first time evidence of a potentiating effect of GH on hepatic porphyrin formation. Our findings demonstrate that HGH increases AIA-induced porphyrin formation in a dose-dependent manner. In contrast HGH had no effect on porphyrin formation from added δ-aminolevulinic acid, suggesting that its potentiating effect on porphyrin formation may be exerted at the level of δ-aminolevulinic acid synthase. Compared to the effect of HGH, an HGH fragment had a much lower stimulatory effect on drug-induced porphyrin formation. It is also known that this NH_2-terminal fragment of HGH retains approximately 10% of the growth-promoting activity of native HGH (20). Importantly, plasmin-modified HGH appeared to have at least the same potency as the native hormone and reduced and alkylated HGH showed a reduced activity. Previous studies demonstrated that plasmin-modified HGH retains full potency with regard to tibial growth-promoting activity and stimulation of hepatic ornithine decarboxylase activity (10, 15, 20). Our present findings suggest that the structural requirements for the effect of growth hormones on hepatic drug-induced porphyrin formation is similar to those required for the induction of tibial growth and hepatic ornithine decarboxylase activity (10, 15, 20). These data also indicate that the observed effect of HGH on porphyrin formation is a consequence of the specific actions of this hormone. The actions of these hormones observed in the present studies are similar also to their effects on erythroid colony formation *in vitro* from murine or human bone marrow stem cells (21), on colony formation from cultured mouse Friend erythroleukemia cells (22) or human K562 erythroleukemia cells (23), and on colony formation from human T-lymphocytes in culture (24).

The potentiating effect of HGH was considerably less than the marked stimulatory effect of insulin (5-fold, Table II) on AIA-induced porphyrin formation. Nevertheless, this effect of growth hormone was reproducible, dose-dependent, and occurred at concentrations as low as 10 ng/ml.

The fact that the effect of HGH could be modified considerably by other hormones, such as cortisol or cAMP, but not by insulin or triiodothyronine, were unexpected in some respects. GH acts synergistically with insulin (12) or with thyroxine (25) and antagonizes some of the effects of cortisol (25) in other experimental systems. The permissive effect of insulin on AIA-induced porphyrin formation, however, is so potent in

TABLE I

Effect of bovine GH, ovine prolactin, HCS, plasmin-treated HGH, and reduced alkylated HGH on AIA-induced porphyrin formation in cultured chick embryo liver cells

Cells were prepared and cultivated as described in Fig. 1. Protoporphyrin IX content was determined after incubation of cell cultures for 24 h in a hormone-supplemented medium containing AIA (100 μg/ml) as described in the text. Data are the mean value ± S.E. for 4 determinations. The control culture with AIA alone formed 26 ± 1 pmol of protoporphyrin IX/mg of protein.

Hormone	Concentration	Protoporphyrin IX formed (mean ± S.E.)
	ng/ml	*% AIA treatment alone*[a]
Bovine GH	10	86 ± 11
	100	86 ± 10
	1000	183 ± 12[b]
Ovine prolactin	100	112 ± 13
	1000	190 ± 13[b]
HCS	100	102 ± 6
	1000	118 ± 33
Plasmin-modified HGH	10	148 ± 17[b]
	100	177 ± 24[b]
	1000	257 ± 21[b]
Reduced alkylated HGH	100	137 ± 13
	1000	149 ± 10[b]

[a] Protoporphyrin IX content of cultures treated with AIA alone = 100%.

[b] $p < 0.05$.

TABLE II

Effect of combined treatment of HGH with other hormones on AIA-induced porphyrin formation

Cells were prepared and cultivated as described in Fig. 1. Cultures were incubated for 24 h in a modified F12 medium supplemented with specific hormones as indicated. Then the medium was replaced with fresh medium containing the same hormone(s) with AIA (100 μg/ml) and cultures were further incubated for 24 h. Protoporphyrin IX content was determined as described in the text. Data are the mean value ± S.E. for 4 determinations.

Hormone	Protoporphyrin IX formed
	pmol/mg protein
None	26.2 ± 0.9
HGH (100)	50.1 ± 3.1
Insulin (500)	139.8 ± 7.1
Insulin (500) + HGH (100)	171.5 ± 18.0
Cortisol (50)	83.9 ± 8.6
Cortisol (50) + HGH (100)	239.5 ± 39.6[a]
Triiodothyronine (100)	58.6 ± 6.1
Triiodothyronine (100) + HGH (100)	69.3 ± 4.9
$N^6,O^{2'}$-Dibutyryl cyclic AMP (2 mM)	67.7 ± 9.6
$N^6,O^{2'}$-Dibutyryl cyclic AMP (2 mM) + HGH (100)	149.6 ± 12.4[a]
8-Bromocyclic AMP (2 mM)	89.5 ± 7.6
8-Bromocyclic AMP (2 mM) + HGH (100)	134.5 ± 2.7[a]

[a] $p < 0.05$ between cultures with and without HGH.

our system that the smaller effect of HGH may not have been detectable. Moreover, the additive effect of insulin and HGH in murine erythroleukemia cells (22) was observed only at very low concentrations of both hormones. The absence of an additive effect between triiodothyronine and HGH may also be due to the maximal dose of the triiodothyronine used in this study. Our findings clearly indicate a synergistic relationship between HGH and cortisol or cAMP on porphyrin formation in cultured chick embryo liver cells, and provide further evidence for the importance of hormone interactions in the regulation of porphyrin formation.

These findings demonstrate a hitherto unrecognized effect of HGH on porphyrin formation in the liver. The use of cells maintained in a serum-free and chemically defined medium shows that this action is not mediated through effects on other tissues. This system is quite useful for defining the mechanism of action of hormones on liver parenchymal cell functions.

Acknowledgments—We wish to thank Drs. A. Kappas and K. E. Anderson for their helpful suggestions. The secretarial assistance of Michele Bifano is also gratefully acknowledged.

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