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SHORT COMMUNICATION

Effect of Varying the Insulin to Glucagon Ratio on Porphyrin Biosynthesis in Chick Embryo Liver Cells

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SUMMARY

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Allylisopropylacetamide-induced porphyrin accumulation in chick embryo liver cells depends upon the insulin to glucagon ratio. A low level of porphyrin accumulation occurs at insulin to glucagon ratios similar to those found following glucose administration *in vivo*, suggesting a possible explanation for the therapeutic effect of glucose in hepatic porphyria.

INTRODUCTION

The administration of glucose *in vivo* has been shown to inhibit the induction by allylisopropylacetamide of δ-aminolevulinic acid synthetase (EC 2.3.1.37) in both rat liver (1) and 17-day-old chick embryo liver (2). Such findings have led to the use of high-carbohydrate diets to suppress acute attacks of hepatic porphyria in humans (3-5). However, several workers have failed to demonstrate a glucose effect on drug-induced δ-aminolevulinic acid synthetase activity *in vitro* (6-8). As the secretion of insulin and glucagon is closely related to the level of blood glucose (9, 10), our objective was to investigate the effects of a range of insulin to glucagon molar ratios on AIA[1]-induced porphyrin biosynthesis in chick embryo liver cells maintained in serum-free culture medium.

Glucagon (bovine and porcine pancreas) and insulin (bovine pancreas, 24 IU/mg) were obtained from Sigma. Chick embryo liver cells were maintained in serum-free Waymouth MD 705/1 medium (5 ml) containing 1 μg/ml of thyroxine (11-13). Insulin was dissolved in acidified 0.15 M NaCl (pH 4), and glucagon, in alkaline 0.15 M NaCl (pH 9). Ten microliters of insulin solution, glucagon solution, or both were present during both 24-hr incubation periods. AIA (15 μg) was dissolved in 10 μl of 95% redistilled ethanol and was added during the second incubation period only. Porphyrins were assayed as previously described (12).

Glucagon enhanced AIA-induced porphyrin biosynthesis in chick embryo liver cells maintained in serum-free medium supplemented with thyroxine, but no other hormones. The maximal stimulatory effect of glucagon occurred at 30 ng/ml (Fig. 1). Insulin was less effective than glucagon (Fig. 2); neither hormone affected porphyrin biosynthesis in the absence of AIA. The addition of insulin but not of glucagon resulted in a small but significant increase of the protein content of the cells attached to the dish.

The effects of varying the insulin to glucagon ratio on AIA-induced porphyrin biosynthesis in chick embryo liver cells main-

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[1] The abbreviation used is: AIA, allylisopropylacetamide.

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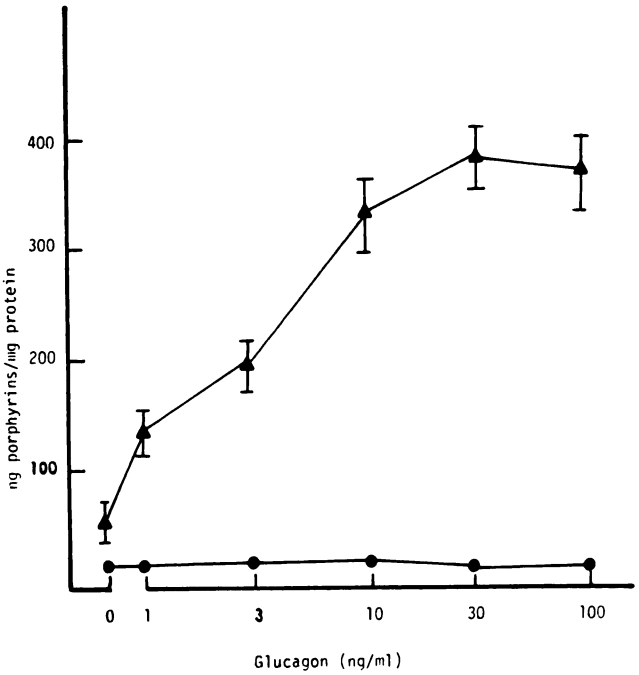

FIG. 1. *Porphyrin accumulation by chick embryo liver cells in response to increasing doses of glucagon*

Cells were maintained in Waymouth MD 705/1 medium supplemented with thyroxine (1 μg/ml) in the presence (▲) and absence (●) of AIA, 3 μg/ml of medium. Each point represents the mean ± standard error of eight determinations.

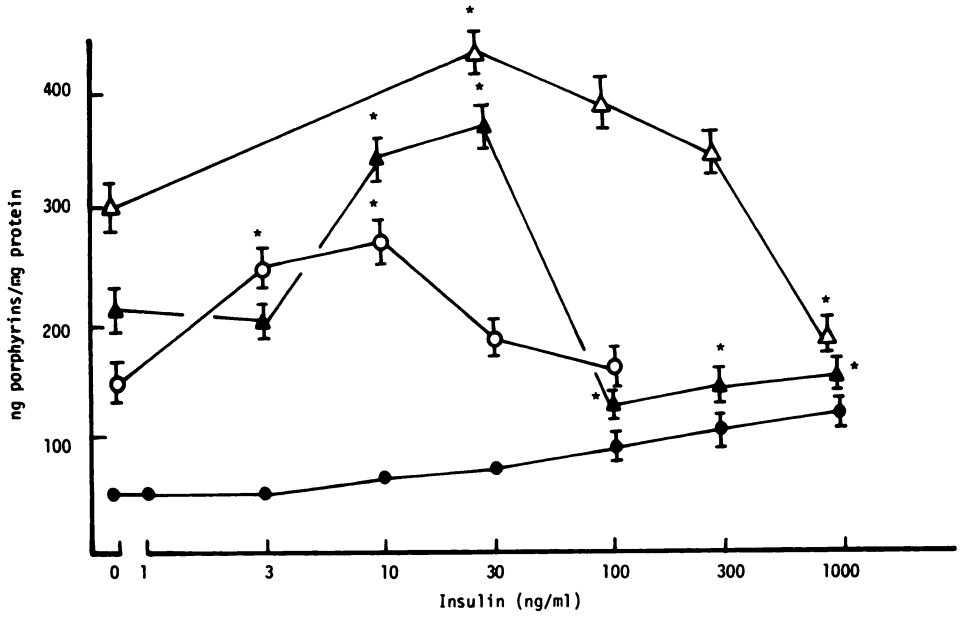

FIG. 2. *Porphyrin accumulation in response to increasing doses of insulin*

●, in the presence of AIA, 3 μg/ml; ○, in the presence of AIA, 3 μg/ml, and glucagon, 1 ng/ml; ▲, in the presence of AIA, 3 μg/ml, and glucagon, 3 ng/ml; △, in the presence of AIA, 3 μg/ml, and glucagon, 30 ng/ml. Each point represents the mean ± standard error of four to eight determinations.

* Significant difference ($p < 0.05$) in porphyrin accumulation between cells maintained in the presence of AIA and both hormones and those maintained in the presence of AIA and glucagon.

tained in serum-free medium containing thyroxine was explored. The response to increasing concentrations of insulin in the presence of 3 μg/ml of AIA and in the presence of constant amounts of glucagon (1, 3, and 30 ng/ml) is shown in Fig. 2. In the presence of glucagon at 1 ng/ml, porphyrin accumulation in response to AIA increased as insulin levels were increased from 0 to 10 ng/ml. At higher concentrations of insulin (30–100 ng/ml), porphyrin accumulation decreased to levels similar to those observed in the absence of insulin. Increasing insulin concentrations to 30 ng/ml in the presence of 3 ng/ml of glucagon resulted in an increase in porphyrin accumulation by AIA compared with that in the absence of insulin. Increasing insulin concentrations further (100–1000 ng/ml) depressed porphyrin accumulation compared with that in the presence of glucagon (3 ng/ml) alone. AIA-induced porphyrin accumulation in the presence of 30 ng/ml of glucagon and 30 ng/ml of insulin was higher than that in the presence of glucagon (30 ng/ml) alone. Increasing insulin (100–1000 ng/ml) levels in the presence of glucagon (30 ng/ml) decreased AIA-induced porphyrin biosynthesis. At 1000 ng/ml of insulin, porphyrin accumulation was lower than in the presence of glucagon (30 ng/ml) alone.

Previous workers (9, 10) have determined plasma insulin to glucagon molar ratios in humans following deprivation of food, ingestion of a balanced meal, and glucose administration (oral and intravenous). The results shown in Fig. 2 were therefore replotted (Fig. 3) as insulin to glucagon molar ratios to facilitate comparison with previous work (9, 10). The molar ratios were calculated using the formula [insulin (microunits per milliliter)/glucagon (picograms per milliliter)] × 23.3 (9). Insulin to glucagon molar ratios of 0.4–1.6, where porphyrin biosynthesis was either maximal or increasing, correspond to those found in human plasma following deprivation of food (9, 10). An insulin to glucagon molar ratio of 3.8, where porphyrin biosynthesis was maximal or beginning to decrease, corresponds to that found in humans following

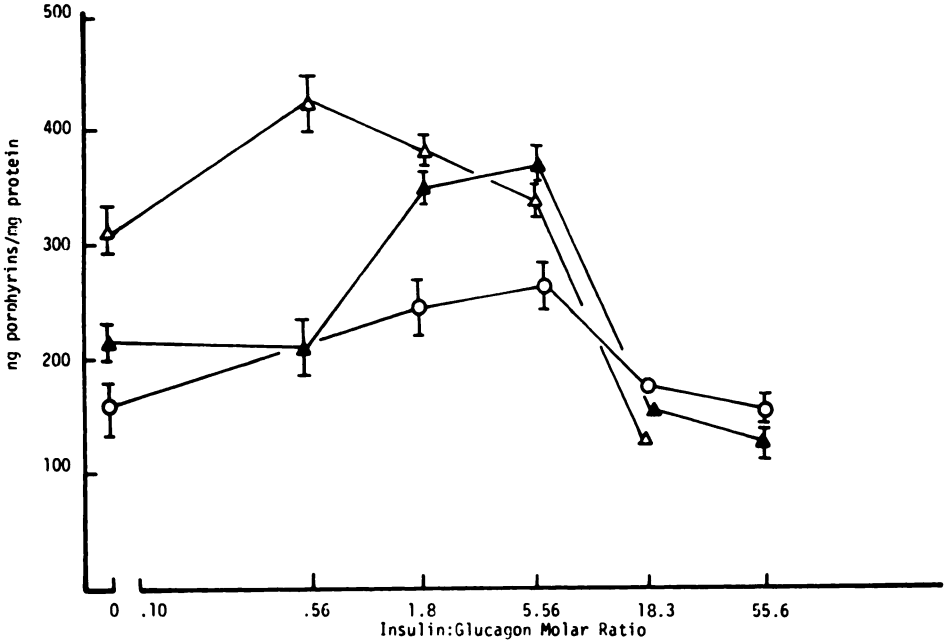

FIG. 3. *Porphyrin accumulation in response to AIA (3 μg/ml) and to increasing insulin to glucagon molar ratios in the presence of a constant glucagon concentration*

○, glucagon, 1 ng/ml; ▲, 3 ng/ml; △, 30 ng/ml. Each point represents the mean ± standard error of four to eight determinations.

ingestion of a balanced meal (9, 10). Insulin to glucagon molar ratios of 16–18, where porphyrin accumulation was markedly depressed, correspond to those found in humans following intravenous glucose administration. Insulin to glucagon molar ratios greater than 18 correspond to those found in humans following ingestion of a high-carbohydrate meal (10) and resulted in marked depression of AIA-induced porphyrin accumulation.

Glucagon and insulin are believed to play an important role in the control of liver metabolism, since the pancreas releases these hormones into the hepatic portal system (14). Glucagon activates hepatic adenylate cyclase, thereby increasing the liver content of cyclic 3′,5′-AMP (14–16). Insulin does not lower the basal level of cyclic AMP within the hepatocyte but antagonizes the glucagon-mediated cyclic AMP elevation (14–16).

Cyclic AMP is essential for the induction of δ-aminolevulinic acid synthetase by AIA in isolated rat hepatocytes (6) and enhances AIA-induced porphyrin biosynthesis in chick embryo liver cell culture (17). It has been suggested that the effects of glucose administration *in vivo* on drug-induced δ-aminolevulinic acid synthetase activity are related to elevation of insulin and reduction of glucagon levels in blood, with a concomitant decrease in hepatic cyclic AMP levels (6). The ascending limbs of the curves in Figs. 2 and 3 may be attributed to the fact that insulin exerts at least two effects in the chick embryo liver cells. Insulin, when added to cells in the absence of glucagon, has a small stimulatory effect on AIA-induced porphyrin biosynthesis, which may be due to its ability to maintain ribosomal integrity (18). Thus it is not surprising that an additive stimulatory effect of insulin and glucagon might be observed at low insulin to glucagon ratios and thus explain the ascending limbs of the curves (Figs. 2 and 3). When the insulin concentration and insulin to glucagon ratio rise, the stimulatory effect of insulin on AIA-induced porphyrin biosynthesis is counteracted by the antagonistic effect on the glucagon-mediated rise in cyclic AMP levels.

We have shown that AIA-induced porphyrin biosynthesis in chick embryo liver cells is highest at low insulin to glucagon molar ratios (2–6), while it is depressed at higher ratios (greater than 16). At insulin to glucagon molar ratios less than 6, hepatic cyclic AMP levels are maximally elevated in rats (15), while at ratios greater than 16, cyclic AMP levels are depressed (15). Therefore the ratio of these two hormones and their control of hepatic cyclic AMP levels may offer a rational explanation for the glucose repression of drug-induced δ-aminolevulinic acid synthetase in laboratory animals *in vivo* (1, 2) and for the therapeutic effect of a high-carbohydrate diet in patients with hereditary hepatic porphyria (3–5).

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REFERENCES

1. Bonkowsky, H. L., Collins, A., Doherty, J. M. & Tschudy, D. P. (1973) *Biochim. Biophys. Acta*, **320**, 561–576.
2. Korinek, J. & Moses, H. L. (1973) *Fed. Proc.*, **32**, 865.
3. Welland, F. H., Hellman, E. S., Gaddis, E. M., Collins, A., Hunter, G. W., Jr. & Tschudy, D. P. (1964) *Metab. (Clin. Exp.)*, **13**, 232–250.
4. Felsher, B. F. & Redeker, A. G. (1964) *Medicine*, **46**, 217–226.
5. Perlroth, M. G., Tschudy, D. P., Marver, H. S., Merard, C. W., Zeigel, R. G., Rechcigl, M. & Collins, A. (1966) *Am. J. Med.*, **41**, 149–162.
6. Edwards, A. M. & Elliott, W. H. (1974) *J. Biol. Chem.*, **249**, 851–855.
7. Bock, K. W., Weiner, R. & Schultz, J. (1976) *Enzyme*, **21**, 488–494.
8. Tomita, Y., Ohashi, A. & Kikuchi, G. (1976) *Tohoku J. Exp. Med.*, **120**, 239–250.
9. Unger, R. H. (1972) *Isr. J. Med. Sci.*, **8**, 252–257.
10. Cahill, G. F., Jr. (1976) in *Peptide Hormones* (Parsons, J. A., ed.), pp. 85–103, University Park Press, Baltimore.
11. Goodridge, A. G. (1975) *Fed. Proc.*, **34**, 117–123.
12. Fischer, P. W. F., Morgan, R. O., Krupa, V. & Marks, G. S. (1976) *Biochem. Pharmacol.*, **25**, 687–693.
13. Morgan, R. O., Fischer, P. W. F., Stephens, J. K. & Marks, G. S. (1976) *Biochem. Pharmacol.*, **25**, 2609–2612.
14. Exton, J. H. & Park, C. R. (1972) in *Handbook of Physiology* (Greep, R. O. & Astwood, E. B.,

eds.), Vol. 1, Sect. 7, pp. 437–455, American Physiological Society, Washington, D.C.
15. Seitz, J. H., Müller, M. J., Nordmeyer, P., Krone, W. & Tarnowski, W. (1976) *Endocrinology*, **99**, 1313–1318.
16. Pilkis, S. J., Claus, T. H., Johnson, R. A. & Park, C. R. (1975) *J. Biol. Chem.*, **250**, 6328–6336.
17. Morgan, R. O., Stephens, J. K., Fischer, P. W. F. & Marks, G. S. (1977) *Biochem. Pharmacol.*, **26**, 1389–1394.
18. Schwarz, A. G. & Amos, H. (1968) *Nature*, **219**, 1366.