AN IN VITRO MODEL DEMONSTRATING THE GLUCOSE EFFECT AND THE INFLUENCE OF FASTING ON PORPHYRIN METABOLISM

Nili SCHOENFELD, Yehudit GREENBLAT, Orit EPSTEIN, Meir LAHAV, Yitzhak BEIGEL and Abraham ATSMON*

The Laboratory of Biochemical Pharmacology and The Department of Internal Medicine B, The Beilinson Medical Center, Petah Tiqva and the Sackler School of Medicine, The Tel Aviv University, Ramat Aviv, Israel

Received 7 December 1981

1. Introduction

Induction of experimental porphyria in laboratory animals by administration of a fast-acting porphyrinogenic drug such as AIA or DDC takes place only when the animal is in the fasting state. The administration of various carbohydrates counteracts the porphyrinogenic effects of these drugs [1–3]. This phenomenon was termed the 'glucose effect' [2], in accordance with a similar effect of glucose on the synthesis of some bacterial enzymes [4]. In contrast to these observations in vivo, no glucose effect could be elicited in vitro in monolayers of chick embryo liver cells ([5], personal communications) and in suspensions of rat liver cells [6].

The aim of this study was to establish an in vitro model to investigate further the influence of fasting and the glucose effect on heme and porphyrin metabolism.

2. Materials and methods

Suspensions of chick embryo liver cells were prepared according to [7]. The final pellets were resuspended in 100 vol. modified Ham's F-12 with insulin, triiodothyronine and hydrocortisone [7]. Monolayers were prepared in 9 cm diameter plastic tissue culture dishes purchased from Nunclon (Roskilde), 9 ml of suspension in each dish. After 24 h incubation in humidified 95% air, 5% CO_2 at 37°C, the medium was replaced by 9 ml modified Earle's medium without glucose (MEBS). MEBS contained per liter: 0.2 g $CaCl_2$, 0.4 g KCl, 0.2 g $MgSO_4 \cdot 7\ H_2O$, 6.8 g NaCl, 2.2 g $NaHCO_3$, 0.14 g $NaH_2PO_4 \cdot H_2O$, 1 mg insulin, 1 mg triiodothyronine, 50 μg hydrocortisone, 100 000 units penicillin and 100 units streptomycin. After 30 min incubation at 37°C, the medium was replaced by fresh MEBS, and the cultures were further incubated for various periods of time. The time of the second exchange of medium with MEBS was regarded as zero time.

ALAS activity in the cells was determined by the direct method [8]. Porphyrins in the culture medium and in the cells were determined as in [5]. Heme was determined by the hot oxalic acid method [7,9]. Proteins were measured as in [10] with bovine serum albumin as standard. Statistical evaluations were made according to Student's *t*-test.

3. Results

A time curve of the activity of ALAS in monolayers of chick embryo liver cells incubated with Ham's F-12 and hormones did not differ from that obtained under the same conditions but without glucose in the medium (not shown).

Preliminary experiments showed that changing the medium with MEBS (modified Earle's medium, without glucose; section 2) caused a rapid rise in ALAS activity. Optimising the conditions for obtaining this effect it was found that a 'preincubation' with MEBS during 30 min further increased ALAS activity. Preincubation in MEBS for 60 min or two 30 min preincubations with MEBS caused no further changes (not shown).

Abbreviations: ALAS, δ-aminolevulinate synthase; AIA, allylisopropylacetamide; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; ALA, δ-aminolevulinic acid

* To whom correspondence should be addressed

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

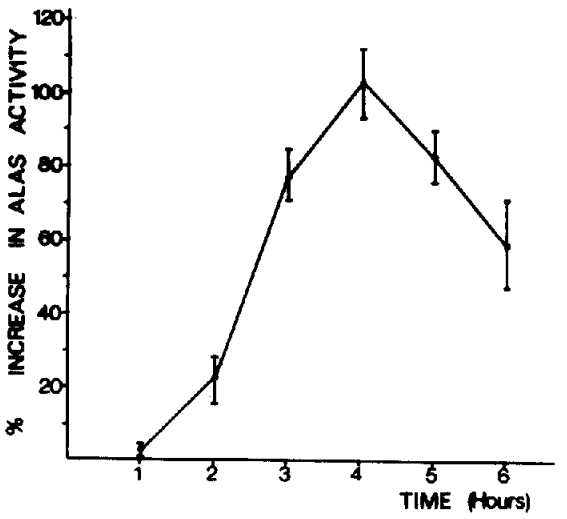

Fig.1. Effects of fasting on ALAS activity in monolayers of chick embryo liver cells. Monolayers of chick embryo liver cells were prepared and 'fasted' in MEBS for different periods of time at the end of which ALAS activity was determined (section 2). Control cells were handled as 'fasted' cells but the medium used was Ham's F-12. At each time point ALAS activity/mg protein of the control cells was considered as 100%. The exchanges of medium did not significantly influence ALAS activity in the control cells. The data shown are each the mean and SD obtained from 6 determinations in 3 separate expt.

Fig.1 shows the time course of ALAS activity after 2 exchanges with MEBS medium with a preincubation of 30 min after the 1st exchange. Maximal ALAS activity was twice that of control cultures with Ham's F-12 ($p < 0.001$). This peak activity occurred generally 4 h after the 2nd change of medium. The decline in ALAS activity after 4 h is most probably related to a decreasing viability of the cells in MEBS as determined by the trypan blue exclusion test. After 24 h incubation in MEBS ~50% of the cell nuclei were stained.

No significant change in the concentrations of either cellular porphyrins or heme could be detected during 6 h of 'fasting' of the monolayers (not shown).

The effects of addition of glucose to MEBS and of porphyrinogenic drugs to MEBS, with and without glucose, are shown in table 1 and compared to their effects on cells incubated in Ham's F-12.

The addition of glucose, 1 mg/ml (5.5 mM) to the MEBS prevented the increase in ALAS activity ($p < 0.001$), demonstrating a glucose effect in this system.

The addition of phenobarbitone up to 250 μg/ml, did not influence ALAS activity during 'fasting' ($p < 0.1$), i.e., incubation in MEBS. After 4 h incubation in the presence of AIA the ALAS activity of cells with MEBS was twice that of cells incubated and induced in Ham's F-12 ($p < 0.001$), and nearly 6 times ($p \ll 0.001$) that of non-induced cells grown in the latter medium. Adding glucose to MEBS partially prevented the induction by AIA but ALAS activity was still clearly above that of the values obtained with Ham's F-12 ($p < 0.02$). DDC, 15 μg/ml, in MEBS enhanced the ALAS activity above that of DDC in Ham's F-12 ($p < 0.001$) and glucose had an inhibitory effect on the induction ($p < 0.001$). Thus, a glucose effect was also observed in cells induced by AIA or DDC.

Table 1
The influence of fasting and of glucose on ALAS activity in non-induced and induced monolayers of chick embryo liver cells

Media	ALAS activity (nmol ALA . mg protein^{-1}. 30 min^{-1})		
	Ham's F-12	MEBS	MEBS + 1 mg/ml Glucose
No addition	0.27 ± 0.05	0.55 ± 0.06	0.29 ± 0.04
+Phenobarbitone (250 μg/ml)	0.26 ± 0.05	0.45 ± 0.05	0.27 ± 0.04
+AIA (60 μg/ml)	0.71 ± 0.16	1.54 ± 0.33	1.03 ± 0.27
+DCC (15 μg/ml)	0.70 ± 0.10	0.96 ± 0.01	0.77 ± 0.09

Monolayers of chick embryo liver cells were prepared as in section 2. After 24 h of incubation the medium, Ham's F-12 with hormones, was replaced by the various media shown. Following a preincubation period of 30 min the media were replaced with fresh ones as shown above. At this time the various substances outlined in the table were added to the cultures. After an additional 4 h incubation ALAS activity of the monolayers was examined. The data shown are each the mean and SD obtained from 8 determinations in 4 separate expt

4. Discussion

Fasting is known to be a causative factor in acute attacks of hepatic porphyrias in patients with latent porphyria [11–14]. A high glucose intake ameliorates the clinical and biochemical signs and symptoms of the acute attack in many patients [14–16]. A similar phenomenon, first described in rats was termed the 'glucose effect' [2]. Subsequently, it was shown that other carbohydrates have also a marked effect on porphyrin metabolism in humans with hepatic porphyria both in the latent phase and during attacks [12–17].

Although the influence of fasting and the glucose effect were also obtained in rats [2] understanding of these phenomena was hampered by the lack of an in vitro model. To the best of our knowledge this is the first time that an in vitro model, demonstrating the effects of fasting and of glucose on porphyrin metabolism, is described. It was shown that ALAS activity in monolayers of chick embryo liver cells is increased after a short incubation in a medium which does not contain substances commonly employed in biological media such as glucose, amino acids, vitamins and others. The addition of glucose to this medium prevents this increase, thus demonstrating the glucose effect. ALAS activity of 'starved' cells induced by AIA or DDC was higher than that of induced cells in Ham's F-12. The glucose effect could also be demonstrated under these conditions. Phenobarbitone did not have a synergistic effect in the system.

The demonstration of a glucose effect in an in vitro system has been attempted by deleting glucose from the incubation medium [5,6]. In view of the results obtained here, the various incubation media generally used for monolayers and suspensions of cells contain substances which have a glucose-like effect. Therefore, all previous in vitro investigations on perturbations in porphyrin and heme metabolism were, probably, carried out under conditions which activated the glucose effect.

In the simple in vitro system described here, the external milieu of the cells can be rigidly controlled. It would, therefore, seem to be eminently suitable for further investigating the influence of carbohydrates on porphyrin metabolism.

References

[1] Rose, J. A., Hellman, E. S. and Tschudy, D. P. (1961) Metabolism 10, 514–521.
[2] Tschudy, D. P., Welland, F. H., Collins, A. and Hunter, G. W. jr (1964) Metabolism 13, 396–406.
[3] Bonkowsky, H. L., Collins, A., Doherty, J. M. and Tschudy, D. P. (1973) Biochim. Biophys. Acta 320, 561–576.
[4] Magasanik, B. (1961) Symp. Quant. Biol. 26, 249–256.
[5] Granick, S. (1966) J. Biol. Chem. 241, 1359–1375.
[6] Edwards, M. and Elliott, W. H. (1974) J. Biol. Chem. 249, 851–855.
[7] Sassa, S. and Kappas, A. (1977) J. Biol. Chem. 252, 2428–2436.
[8] Sinclair, P. and Granick, S. (1977) Anal. Biochem. 79, 380–393.
[9] Morrison, G. R. (1965) Anal. Chem. 37, 1124–1126.
[10] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[11] Knudsen, K. B., Sparberg, M. and Lecocq, F. (1967) New Engl. J. Med. 277, 350–351.
[12] Felsher, B. F. and Redecker, A. G. (1967) Medicine 46, 217–223.
[13] Perlroth, M. G., Tschudy, D. P., Ratner, A., Spahr, W. and Redeker, A. (1968) Metabolism 17, 571–581.
[14] Stein, J. A. and Tschudy, D. P. (1970) Medicine 49, 1–16.
[15] Bonkowsky, H. L., Magnussen, C. R., Collins, A. R., Doherty, J. M., Hess, R. A. and Tschudy, D. P. (1976) Metabolism 25, 405–414.
[16] Brodie, M. J., Moore, M. R., Thompson, G. G. and Goldberg, A. (1977) Clin. Sci. Mol. Med. 53, 365–371.
[17] Welland, F. H., Hellman, E. S., Gaddis, E. M., Collins, A., Hunter, G. W. and Tschudy, D. P. (1964) Metabolism 13, 232–250.