EFFECTS OF INDUCER SUBSTANCES ON PORPHYRIN SYNTHESIS IN LIVER CELL CULTURES

Manfred DOSS
Hygiene-Institut der Philipps-Universität, 355 Marburg an der Lahn, Germany

Received 18 November 1968

1. Introduction

The application of sedormid [1], hexachlorbenzene [2] and 3,5-dicarbethoxy-1,4-dihydrocollidine [3] to healthy animals induced the biochemical manifestations of hepatic porphyria. Granick and Kappas [4–6] showed that foreign chemicals, certain drugs, and 5β-H-steroid metabolites from the biotransformation of sex hormones stimulated porphyrin synthesis in chick-embyo liver cells growing in culture. Certain steroids also induced enhanced formation of hemoglobin in erythroid cells of chick blastoderm cultured *in vitro* [7]. Regulative enzymes of the heme biosynthetic pathway are thought to include δ aminolevulinic acid synthetase [4] and succinyl-CoA synthetase [8] in the liver, δ aminolevulinic acid dehydratase [9] in the bacterium Rhodopseudomonas spheroides and heme synthetase [10] in human bone marrow.

This communication reports on the biosynthesis of the individual porphyrins, not yet differentiated by others [4,5], in primary chick-embryo liver cell cultures under the influence of 2-methyl-2-*n*-propyl-1,3-propandioldicarbamate and of 5β-androstane-3α-ol,17-one.

2. Experimental procedure

The livers of 17-day old chick embryos [4] were reduced to small pieces, using known techniques [11] under sterile conditions, washed three times in Hank's balanced salt solution [12] at 4°C. Cells were dispersed by 0.2% trypsin solution at 37°C. After sedimentation (+4°C, 50 × *g*, 3 min) the cells were enumerated by counting crystal violet-stained nuclei, suspended in growth medium and diluted to 10^6 liver cells/ml. The further procedure is outlined in the legend of fig. 1. At least four cultures were pooled for chemical analysis at the various time intervals.

The porphyrins were separated and isolated as methylesters by thin-layer chromatography [14] on pre-coated silica gel plates in a one-dimensional, threefold development system [15]. Protohemin methylester was converted into the pyridine hemichrome [15] and measured by difference-spectrum [16]. The porphyrin methylesters, eluted from the adsorbens, were converted into copper chelates for spectrophotometric analysis [17]. For determination of porphyrin concentrations about 1–2 pmoles/10^6 liver cells the extracts of several cultures were pooled for chromatography.

3. Results

The results of typical experiments are given in figs. 1 and 2. The concentration of protoporphyrin first arose. Three hours after the elevation of protoporphyrin, coproporphyrin and, some hours later, uroporphyrin increased. This principle of stimulating the increase of porphyrins was found in all cultures to which a very active drug or steroid inducer was added. The effects of both tested substances was significant ($p = 0.01$). The control cultures showed merely unimportant alterations of porphyrin concentrations within the period of cultivation. When hemin was added in concentration of 10^{-5} M the increase of porphyrins was strongly inhibited. From cultures

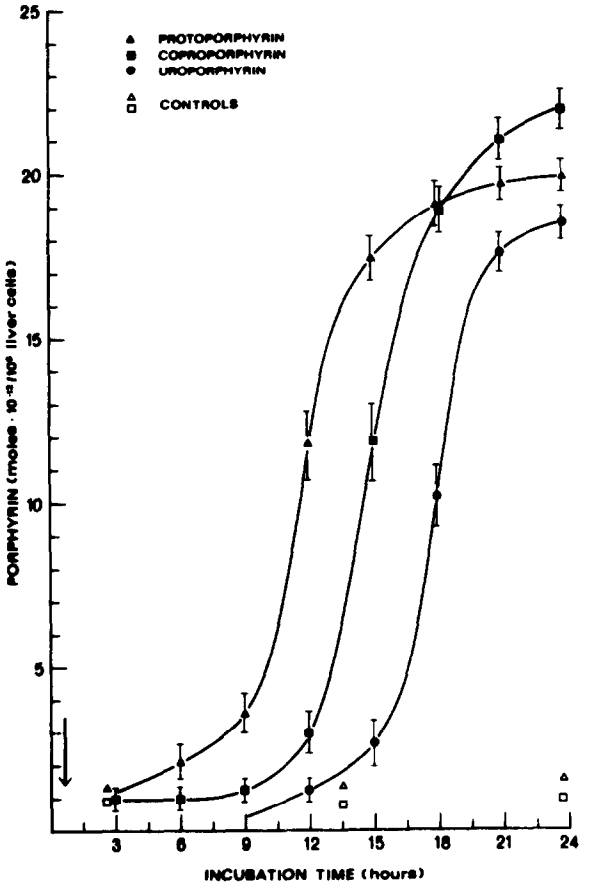

Fig. 1. Biosynthesis of proto-, copro- and uroporphyrin in chick-embryo liver cells growing in culture after induction with 2-methyl-2-*n*-propyl-1,3-propandioldicarbamate (200 μg/ml medium). Chick-embryo liver cells were incubated in Petri dishes (diameter 5 cm) at 37°C in 5% CO_2-air. One culture contained 5 X 10^6 hepatic cells in 5 ml of growth medium (Eagle's medium containing antibiotics, supplemented with 10% calf serum and 0.5% lactalbumin hydrolysate). The medium was changed after 20–24 hr and the inducer substance, dissolved in 3 μl of absolute ethanol, was then added (↓). Analyses of porphyrins were made from the lyophilized cultures (cells plus medium). Uroporphyrin could not be measured in the controls. The graphs indicate the average deviation from the mean.

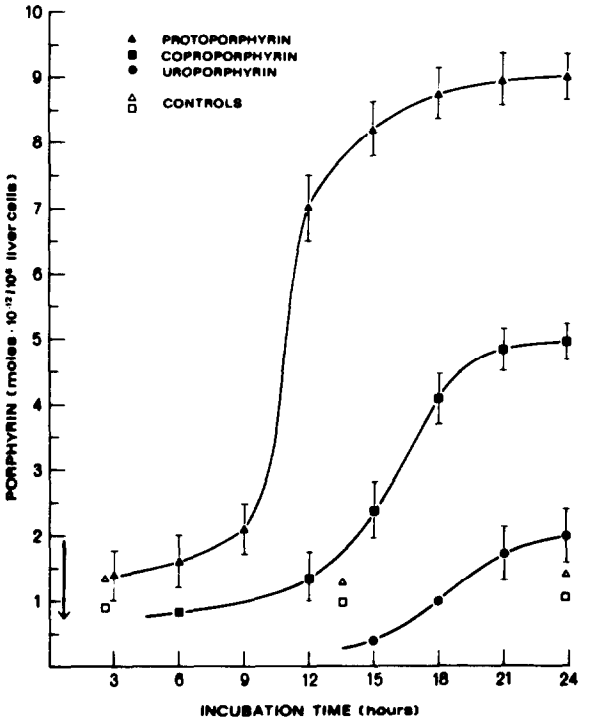

Fig. 2. Biosynthesis of porphyrins in chick-embryo liver cells growing in primary culture after induction with 5β-androstane-3α-ol,17-one (5 μg/ml medium). The steroid was added (↓) after the medium was replaced by 5 ml of fresh growth medium. For incubation conditions and determination of porphyrins see legend to fig. 1 and experimental procedure.

treated with other steroids of the 5β-H (A:B cis-type ring) configuration, it was evident that the porphyrin primarily formed by the inducing process was protoporphyrin, in comparison to the porphyrins with three to eight carboxyl groups. Comparatively weak inducing substances (e.g. estradiol) in optimal amounts for induction or the most potent inducers in lower concentrations led to an increase of protoporphyrin only in the liver cells. This is characteristic for both drugs of several chemical groups and for steroids. The enhancement of protoporphyrin is a parameter for the intensity of the stimulating effect on porphyrin synthesis.

For close identification greater amounts of porphyrins were needed than those encountered in most of the cultures described. For this purpose δ-aminolevulinic acid (10^{-4} M) was added to some cultures and the synthetized porphyrins were isolated as pure substances [14]. The R_f-values of proto-, copro- and uroporphyrin in several solvent systems [14,15], the adsorption maxima of the esters in chloroform [14, 18] and of their Cu-chelate complexes [17], and also the fluorescence excitation and emission spectra

were identical with the behaviour of reference substances. The dicarboxy-porphyrin methylester with chromatographic and spectral data of protoporphyrin IX was converted both in protohemin by the ferrous sulfate method [19] and in hematoporphyrin IX [20]. The products were analysed and identified as reported recently [15,20].

4. Discussion

In the view of Granick [4] the inducers mentioned in the introduction act as derepressors for the rate limiting enzyme in heme synthesis: δ-aminolevulinic acid synthetase.

In addition to protoporphyrin heme was also still elevated in cultures treated with 2-methyl-2-*n*-propyl-1,3-propandioldicarbamate and 3,5-dicarbethoxy-1,4-dihydrocollidine compared with controls after an incubation period of 24 hr [15]. But at this time all cultures exhibit lower heme content in comparison with the first few hours of incubation. Since heme content in the control cultures during the incubation also decreased, the question remains open, whether in the *in vitro* system heme is really synthetized from protoporphyrin formed by the inducing process. In regard to the marked enhancement of protoporphyrin synthesis after induction mitochondrial coproporphyrin oxidase [21,22] is active under the conditions of liver cell cultivation. The fact that protoporphyrin, the end product of the porphyrin chain, rises first, but the increase of copro- and uroporphyrin comes only three to six hours later, might be due to some cellular end product regulatory mechanism, the nature of which has not yet been studied.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg. The author thanks Dr. F.Lehmann-Grube, Marburg, for generous help in the cell culture technique, Prof. Th.Bücher, München, and Prof. P. Karlson, Marburg, for valuable discussions of the manuscript.

References

[1] R.Schmid and S.Schwartz, in: Ciba Foundation Symposium on Porphyrin Biosynthesis and Metabolism (J. & A. Churchill Ltd., London, 1955) p. 196.
[2] R.K.Ockner and R.Schmid, Nature 189 (1961) 499.
[3] S.Granick and G.Urata, J. Biol. Chem. 238 (1963) 821.
[4] S.Granick, J. Biol. Chem. 241 (1966) 1359.
[5] S.Granick and A.Kappas, J. Biol. Chem. 242 (1967) 4587.
[6] A.Kappas and S.Granick, J. Biol. Chem. 243 (1968) 346.
[7] R.D.Levere, A.Kappas and S.Granick, Proc. Natl. Acad. Sci. U.S. 58 (1967) 985.
[8] R.F.Labbe, T.Kurumada and J.Onisawa, Biochim. Biophys. Acta 111 (1965) 403.
[9] J.Lascelles, J. gen. Microbiol. 23 (1960) 487.
[10] S.S.Bottomley, Blood 31 (1968) 314.
[11] F.Lehmann-Grube, in: Virus- und Rickettsieninfektionen des Menschen (J.F.Lehmanns Verlag, München, 1965) pp. 30–65.
[12] J.H.Hanks and R.E.Wallace, Proc. Soc. Exp. Biol. Med. 71 (1959) 196.
[13] H.Eagle, Science 130 (1959) 432.
[14] M.Doss, J. Chromatography 30 (1967) 265.
[15] M.Doss, Klin. Wschr. 46 (1968) 731.
[16] R.J.Porra and O.T.G.Jones, Biochem. J. 87 (1963) 181.
[17] M.Doss, Z. Klin. Chem. Klin. Biochem., in press.
[18] J.E.Falk, Porphyrins and Metalloporphyrins (Elsevier, Amsterdam, 1964) p. 232.
[19] J.E.Falk, idem, p. 130.
[20] M.Doss and H.Bürger, Hoppe-Seyler's Z. Physiol. Chem. 348 (1967) 936.
[21] S.Sano and S.Granick, J. Biol. Chem. 236 (1961) 1173.
[22] R.J.Porra and J.E.Falk, Biochem. J. 90 (1963) 69.