Volume 233, number 1, 47–50 FEB 05902 June 1988

Periportal and perivenous hepatocytes respond equally to glycogenolytic agonists

Stefaan Keppens and Henri De Wulf

Afdeling Biochemie, Campus Gasthuisberg, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

Received 19 March 1988

We have used the technique of short-term infusion with digitonin to obtain hepatocytes originating either from the periportal or the perivenous zone of the liver acinus [(1985) Biochem. J. 229, 221–226]. Total glycogen phosphorylase content and sensitivity to cyclic AMP-dependent and calcium-mediated glycogenolytic agonists were very similar for both cell sub-populations and did not differ from the values obtained for control cells. We conclude therefore that there is an apparent absence of metabolic zonation as far as receptor-mediated glycogenolysis and glycogenolytic potency is concerned.

Glycogenolysis; Metabolic zonation; Hormone; (Hepatocyte)

1. INTRODUCTION

The liver performs a variety of key functions in the organism where it is not only responsible for the maintenance of the energy supply but also catalyzes various important biosynthetic and biodegradative processes. As reviewed recently by Jungermann [2] metabolic zonation of the liver is clearly involved in the realization of many of these different functions. Histochemically, different types of hepatocytes have indeed been detected; based on the blood stream, the liver can be considered as being composed of at least two functionally different zones, the periportal and the perivenous zones. The periportal cells, for instance, are better equipped to perform gluconeogenesis whereas perivenous hepatocytes contain more key enzymes for glycolysis. To our knowledge, no data are available concerning the glycogenolytic capacity or regulation of the two sub-populations of hepatocytes. We have therefore undertaken a study to check whether perivenous or periportal hepatocytes would present different glycogenolytic sensitivities towards well established glycogenolytic agonists. We have selected glucagon as the prototype of cyclic AMP-mediated hormones and angiotensin, vasopressin and the α_1-adrenergic agonist, phenylephrine, which are well known to initiate a calcium-dependent glycogenolytic response. ATP was also included since this nucleotide exerts a powerful glycogenolytic effect [3–6] most probably at the level of the P_{2Y}-purinoceptors [7].

Glutamate dehydrogenase (GDH) activity is used as the criterion to decide whether the cell preparations are enriched in perivenous or periportal cells. Indeed, GDH is found predominantly in the perivenous region of the liver acinus, resulting in periportal/perivenous ratio equal to 0.72 [8].

Correspondence address: S. Keppens, Afdeling Biochemie, Campus Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium

2. EXPERIMENTAL

2.1. *Materials*

We used male Wistar-strain albino rats (200–250 g body wt) that were fed ad libitum. Digitonin was from Serva (Heidelberg, FRG) and was rendered water soluble as described by Quistorff [1]. Collagenase was from Sigma (St. Louis, MO); other chemicals were of the purest grade available.

2.1.1. Preparation of hepatocytes originating from the periportal or the perivenous location in the liver acini

We have used the combined digitonin/collagenase perfusion technique described by Quistorff [1] except that the infusion of

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

digitonin was done at 20 mg/ml and was stopped after 20 to 30 s. Periportal cells are obtained when digitonin is infused in the retrograde way (via the vena cava) thereby destroying preferentially the perivenous region of the liver acini. Perivenous cells are obtained after the anterograde (via the portal vein) infusion of digitonin. Control cells are prepared in the same way as perivenous or periportal hepatocytes, except for the use of digitonin. The incubation and sampling of the liver cells were as described by Keppens and De Wulf [9]. Protein concentration was determined by the method of Bradford [10] with bovine serum albumin as standard.

2.2. *Enzyme assays*

GDH activity was measured according to Bergmeyer [11]. Frozen liver biopsies were homogenised in 3 vols of 1.15% KCl and further diluted with water to obtain a final dilution of 1/20. Frozen liver cells were thawed shortly before the assay. Both the liver homogenate and the cell homogenate were treated for 2 to 3 min at room temperature with 0.2% Triton X-100 prior to the GDH assay.

Phosphorylase *a* assay was done as described by Keppens and De Wulf [9]. Total glycogen phosphorylase ($a+b$) activity was determined according to Stalmans and Hers [12].

3. RESULTS AND DISCUSSION

Control cells and hepatocytes, obtained after the selective destruction by digitonin of either the perivenous or the periportal regions of the liver acini, were analysed for their content of GDH ac-

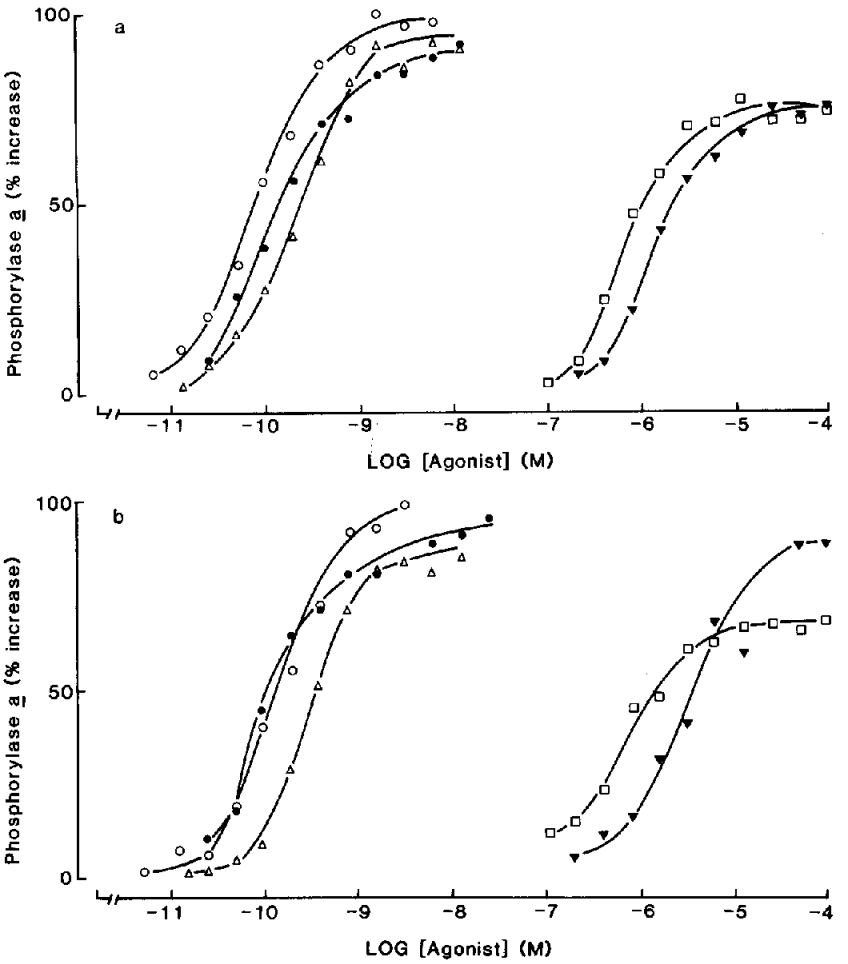

Fig.1. Dose-dependent activation of phosphorylase by different glycogenolytic agonists in periportal and perivenous hepatocytes. Periportal (a) and perivenous (b) hepatocytes were incubated for 1 min with increasing concentrations of glucagon (○), vasopressin (●), angiotensin (△) and phenylephrine (□) or for 20 s with increasing concentrations of ATP (▼). Phosphorylase *a* was then assayed and is expressed as percent increases above control. Values shown are means of 4 to 5 (see table) different experiments. Control and maximally stimulated phosphorylase values range from 20–30 to 70–80 mU/mg protein respectively for both perivenous and periportal cells.

Table 1

K_A values for the glycogenolytic agonists

Cells	Glucagon 10^{-10} M	Vasopressin 10^{-10} M	Angiotensin 10^{-10} M	Phenylephrine 10^{-6} M	ATP 10^{-6} M
Control	1.0 ± 0.4(5)	0.9 ± 0.2(5)	1.2 ± 0.5(5)	0.97 ± 0.4(4)	1.0 ± 0.5(5)
Periportal	1.1 ± 0.2(5)	1.1 ± 0.1(5)	2.2 ± 0.5(5)	0.85 ± 0.2(5)	1.1 ± 0.2(5)
Perivenous	1.6 ± 0.4(5)	1.3 ± 0.2(5)	2.0 ± 0.5(4)	0.76 ± 0.2(4)	1.2 ± 0.3(4)

These values were computed as indicated in the text and are given as means ± SD (n)

tivity. The ratio of GDH activity of a cell suspension over the GDH activity of a biopsy, taken from the same liver before digitonin treatment, is used for the ascertainment of the zonal origin of the cells. For control cells, due to the elimination of inert proteins, the GDH activity ratio (± SD) cell/biopsy was 1.45 ± 0.11(5); for the presumed perivenous and periportal cells, this ratio was 1.68 ± 0.3(6) and 1.05 ± 0.24(5), respectively ($P < 0.05$ by the Student's t-test). This gives a periportal/perivenous activity ratio of 0.62, a clear indication (see section 1) for a selective enrichment in periportal and perivenous hepatocytes.

Cell populations enriched in either perivenous or periportal hepatocytes were preincubated with 10 mM glucose to lower their phosphorylase a content to 20–30 mU/mg of protein in both instances (see legend to fig.1). They were then challenged for 1 min with increasing concentrations of a glycogenolytic agonist, except for ATP where the exposure of the cells was reduced to 20 s. We have shown previously [3,9] that both time intervals are largely sufficient to allow the development of enzyme activation. As is shown in fig.1, each agonist produced a quite similar dose-dependent activation of glycogen phosphorylase in the periportal and perivenous cell preparations. In both cell populations maximal activations are similar albeit somewhat lower with phenylephrine and ATP. K_A values (agonist concentrations at which half maximal activation is obtained) have been computed as described previously [3,13–15]; they are tabulated in table 1, and are compared with control values. No statistically significant differences (as obtained by the Student's t-test) are revealed between the K_A values obtained with periportal or perivenous hepatocytes for a given agonist. Due to the inherent variability, small changes (up to 2-fold) in K_A values might be overlooked. We conclude therefore that perivenous and periportal hepatocytes cannot be distinguished from each other with respect to their receptor-mediated glycogenolysis, whether this process is mediated by cyclic AMP (glucagon) or by calcium ions (phenylephrine, vasopressin, angiotensin and ATP). Total ($a+b$) activity (mU/mg protein ± SD) of glycogen phosphorylase was 96.6 ± 15.0(5) for periportal cells and 90.3 ± 10.8(5) for perivenous cells, indicating that both cell types possess the same glycogenolytic potency.

These data do not however exclude an in vivo occurrence of zonation of hormonal glycogenolysis. Indeed, using a perfused system, Häussinger et al. [16] reported on liver heterogeneity in response to extracellular ATP, the periportal area being more responsive to ATP than the perivenous hepatocytes. These data are not necessarily in contradiction with our findings showing no different glycogenolytic capacity between hepatocytes originating from the two zones. Factors existing in vivo and lost in vitro upon isolation of the hepatocytes, might result in some in situ metabolic zonation. The existence in vivo of hormone, substrate and product gradients [2], the possible influence of hemodynamic changes induced by the agonists tested in vivo are a few examples of differences between in vivo and in vitro conditions.

Acknowledgements: This work was supported by the Belgian FGWO. We thank G. Cumps and J. Schillemans for skillful technical assistance, J. Stuyts for the illustrations and M. Coppens for careful typing of the manuscript.

REFERENCES

[1] Quistorff, B. (1985) Biochem. J. 229, 221–226.
[2] Jungermann, K. (1986) Enzyme 35, 161–180.
[3] Keppens, S. and De Wulf, H. (1985) Biochem. J. 231, 797–799.

[4] Charest, R., Blackmore, P.F. and Exton, J.H. (1985) J. Biol. Chem. 260, 15789–15794.
[5] Sistare, F.D., Picking, R.A. and Haynes, R.C. (1985) J. Biol. Chem. 260, 12744–12747.
[6] Buxton, B.D., Robertson, M.S. and Olson, S.M. (1986) Biochem. J. 237, 773–780.
[7] Keppens, S. and De Wulf, H. (1986) Biochem. J. 240, 367–371.
[8] Lindros, K.O. and Penttilä, K.E. (1985) Biochem. J. 228, 757–760.
[9] Keppens, S. and De Wulf, H. (1984) Biochem. J. 222, 277–280.
[10] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[11] Bergmeyer, H.U. (1983) Methods of Enzymatic Analysis, Verlag Chemie, Weinheim, FRG.
[12] Stalmans, W. and Hers, H.-G. (1979) Eur. J. Biochem. 54, 341–350.
[13] Bréant, B., Keppens, S. and De Wulf, H. (1981) Biochem. J. 200, 509–514.
[14] Cantau, B., Keppens, S., De Wulf, H. and Jard, S. (1980) J. Receptor Res. 1, 137–168.
[15] Keppens, S., De Wulf, H., Clauser, P., Jard, S. and Morgat, J.L. (1982) Biochem. J. 208, 809–817.
[16] Häussinger, D., Stehle, T., Gerok, W., Tran-thi, T. and Decker, K. (1987) Eur. J. Biochem. 169, 645–650.