Effect of inositol and tri-iodothyronine on the hormonal responsiveness of hepatocytes obtained from partially hepatectomized rats

Judith HUERTA-BAHENA and J. Adolfo GARCÍA-SÁINZ*

Departamento de Bioenergética, Centro de Investigaciones en Fisiología Celular and Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Apartado Postal 70-600, 04510 México D.F., México

(Received 9 July 1984/Accepted 30 August 1984)

Hepatocytes obtained from animals partially hepatectomized (72h before the experiment) have a diminished responsiveness to α_1 -adrenergic amines, vasopressin, angiotensin and glucagon and an increased responsiveness to β -adrenergic amines. Administration of inositol or tri-iodothyronine to the hepatectomized animals induced a recovery in the hepatocyte responsiveness to the Ca^{2+} -dependent hormones and abolished that to β -adrenergic amines; the response to glucagon was not improved.

It has been previously observed by us (Huerta-Bahena *et al.*, 1983a) and other authors (Aggerbeck *et al.*, 1983) that, during the process of active proliferation which follows partial hepatectomy, the type of adrenergic receptor which mediates the actions of adrenaline changes dramatically from α_1 -adrenergic in the control hepatocytes to β in regenerating liver. The increased β -adrenergic responsiveness of the newly formed hepatocytes seems to be associated with a marked increase in the number of β -adrenoceptors (Huerta-Bahena *et al.*, 1983a; Aggerbeck *et al.*, 1983). However, the decreased α_1 -adrenergic responsiveness does not seem to be due exclusively to changes in the number of α_1 -adrenoceptors (Huerta-Bahena *et al.*, 1983a; Aggerbeck *et al.*, 1983). The metabolic actions of other hormones that seem to act through a similar process of signal transduction to α_1 -adrenergic amines, such as vasopressin and angiotensin, are also markedly diminished in the newly formed hepatocytes (Huerta-Bahena *et al.*, 1983b). This process of signal transduction is far from completely characterized, but it is accepted that it involves calcium signalling and phosphoinositide breakdown (Michell, 1975; Fain & García-Sáinz, 1980; Williamson *et al.*, 1981). Interestingly, the content of phosphatidylinositol in the newly formed hepatocytes was found to be diminished (Huerta-Bahena *et al.*, 1983a), and it was found that the administration of inositol to the partially

hepatectomized rat induces the recovery of responsiveness of the hepatocytes to α_1 -adrenergic amines, vasopressin and angiotensin (Huerta-Bahena & García-Sáinz, 1983).

Thyroid hormones are known to control the biosynthesis of inositol in the liver of the rat (Hasegawa & Eisenberg, 1981), and it has been observed that after partial hepatectomy the blood concentrations of thyroid hormones decrease markedly (Bucher & Swaffield, 1975). From these data, the hypothesis was made that thyroid hormones may mimic the action of inositol. The effect of the administration of T_3 to partially hepatectomized rats on the responsiveness of their hepatocytes was studied, and the action of T_3 was compared with that of inositol. Our results indicate that thyroid hormones partially restore the responsiveness of the newly formed liver cells to α_1 -adrenergic amines, vasopressin and angiotensin.

Materials and methods

(-)-Adrenaline, [arginine] vasopressin, T_3 , angiotensin, ($\pm$)-propranolol, urease, glucose oxidase and peroxidase were obtained from Sigma Chemical Co. Bovine serum albumin (fraction V) and collagenase (type II) were obtained from Armour and Worthington respectively. Glucagon was generously given by Eli Lilly. Female Wistar rats weighing 180-200g were used. Hepatectomy (excision of approx. 80% of the liver) was performed by routine procedures (Higgins & Anderson, 1931). In order to differentiate the

Abbreviation used: T_3 , 3,3',5'-tri-iodothyronine.

* To whom reprint requests should be addressed.

effects of partial hepatectomy from those of the surgical stress, sham operations (which consisted of incision, digital manipulation of the liver and closure) were performed. No effect of sham operations was observed, so these results are omitted for clarity. T_3 (25 μ g/100g body wt., subcutaneously) or inositol (25mg/100g, intraperitoneally) was administered (between 08:00 and 10:00h) 24 and 48 h after partial hepatectomy.

Hepatocytes were isolated from female Wistar rats (180–200g) by the method of Berry & Friend (1969) as modified by Tolbert *et al.* (1980). Hepatocytes (about 40mg wet wt.) were incubated for 60 min at 37°C in a water-bath shaker in 1 ml of Krebs–Ringer bicarbonate buffer (Tolbert *et al.*, 1980) containing 1% albumin at pH 7.4 under O_2/CO_2 (19:1). Urea production was determined in cells from fed rats in medium supplemented with 2mM-ornithine and 10mM-glutamine, and it was assayed by the method of Gutman & Bergmeyer (1974).

Results and discussion

α -Adrenergic activation (adrenaline + propranolol), vasopressin and angiotensin stimulate ureogenesis in normal rat hepatocytes (Fig. 1). β -Adrenergic activation (isoprenaline) had no effect on these cells (Fig. 2). In contrast, in hepatocytes obtained from rats partially hepatectomized 3 days before the experiment was performed, adrenaline plus propranolol, vasopressin and angiotensin were without effect, and isoprenaline produced a significant ($P < 0.001$) stimulation of ureogenesis (Figs. 1 and 2). Administration of inositol or T_3 significantly increased the responsiveness of the newly formed hepatocytes to α -adrenergic amines, vasopressin and angiotensin, and diminished the β -adrenergic responsiveness of the liver cells (Figs. 1 and 2).

It has been previously reported that hepatocytes obtained from rats partially hepatectomized 7 days before the experiment was performed have a pattern of hormonal responsiveness similar to the

Fig. 1. Effect of the administration of inositol or tri-iodothyronine to partially hepatectomized rats on the responsiveness of their hepatocytes to α -adrenergic activation, angiotensin or vasopressin

Hepatocytes isolated from control animals (○) or animals partially hepatectomized receiving no treatment (●), inositol (■) or T_3 (▲) were incubated in the presence of different concentrations of adrenaline plus 10 μ M-propranolol (a), angiotensin (b) or vasopressin (c). Data are expressed as % of basal urea production, which was: control 22 ± 2 , hepatectomized 44 ± 9 , hepatectomized injected with inositol 25 ± 2 and hepatectomized injected with T_3 21 ± 1 (nmol/mg wet wt. of cells). Plotted are the means $\pm$ S.E.M. of at least four determinations with different cell preparations.

Fig. 2. Effect of the administration of inositol or tri-iodothyronine to partially hepatectomized rats on the responsiveness of their hepatocytes to isoprenaline or glucagon

Hepatocytes were incubated in the presence of different concentrations of isoprenaline or glucagon. Other methods, symbols etc. were as in Fig. 1.

controls (Huerta-Bahena & García-Sáinz, 1983). Therefore the results obtained with cells from rats treated with either inositol or T_3 suggested that these agents were accelerating the differentiation process of the liver. However, a clear difference became apparent when the effect of glucagon was studied. The effect of glucagon is markedly diminished in cells isolated from animals operated 3 days before the experiment was performed, but only slightly diminished in cells obtained 7 days after partial hepatectomy (Huerta-Bahena *et al.*, 1983b). In our present experiments using cells from animals hepatectomized 3 days before the experiments, the administration of either inositol or T_3 did not improve the response of the cells to glucagon (Fig. 2).

Our data indicate that the administration of inositol or T_3 increases the responsiveness of the cells to agents that seem to act through Ca^{2+} and phosphoinositide breakdown (Fig. 1). This effect is accompanied by a decrease in the β -adrenergic responsiveness of the cells. It is not clear at present how these two processes are co-ordinated. However, it is clear that, under these conditions, the recovery of the response to glucagon is dissociated in time from the above-mentioned changes.

Inositol was more effective than T_3 in inducing the recovery of the response to Ca^{2+} -dependent hormones (Fig. 1), and in some cases the response of these cells to the hormones was even greater than that of control cells.

We have suggested the possibility that inositol might be a limiting factor for the hormonal responses (Huerta-Bahena & García-Sáinz, 1983; Huerta-Bahena *et al.*, 1983a,b), i.e. that the newly formed hepatocytes could be deficient in inositol. The data with T_3 , which is known to increase hepatic inositol synthesis, are consistent with such a suggestion. However, it should be stressed that we have been unable to reproduce the effects *in vitro*, i.e. by adding inositol or T_3 to the hepatocytes, and therefore the role of extrahepatic tissues or humoral factors other than inositol or T_3 cannot be ruled out. A very significant advance in our understanding of the role of phosphoinositides in the mechanism of action of the Ca^{2+} -dependent hormones has been achieved in the last few years (Creba *et al.*, 1983; Berridge, 1984; Joseph *et al.*, 1984). However, many links are missing in the chain of events that couple receptor activation to the final effects. Regenerating liver seems to be an attractive model in which to elucidate such events.

This research was partially supported by a Grant from CONACyT (PCCBBNA 020747). We thank Ms. Guadalupe Ramírez for typing the manuscript.

References

- Aggerbeck, M., Ferry, N., Zafrani, E. S., Billon, M. C., Barouki, R. & Hanoune, J. (1983) *J. Clin. Invest.* **71**, 476–486
- Berridge, M. J. (1984) *Biochem. J.* **220**, 345–360
- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–512
- Bucher, N. R. L. & Swaffield, M. N. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1157–1160
- Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H. & Kirk, C. J. (1983) *Biochem. J.* **212**, 733–747
- Fain, J. N. & García-Sáinz, J. A. (1980) *Life Sci.* **26**, 1183–1194
- Gutman, I. & Bergmeyer, H. U. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1791–1794, Academic Press, New York
- Hasegawa, R. & Eisenberg, F. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4863–4866
- Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
- Huerta-Bahena, J. & García-Sáinz, J. A. (1983) *Biochim. Biophys. Acta* **763**, 125–128
- Huerta-Bahena, J., Villalobos-Molina, R. & García-Sáinz, J. A. (1983a) *Biochim. Biophys. Acta* **763**, 112–119
- Huerta-Bahena, J., Villalobos-Molina, R., Corvera, S. & García-Sáinz, J. A. (1983b) *Biochim. Biophys. Acta* **763**, 120–124
- Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. (1984) *J. Biol. Chem.* **259**, 3077–3081
- Michell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Tolbert, M. E. M., White, A. C., Aspary, K., Cutts, J. & Fain, J. N. (1980) *J. Biol. Chem.* **255**, 1938–1944
- Williamson, J. R., Cooper, R. H. & Hoek, J. B. (1981) *Biochim. Biophys. Acta* **639**, 243–295