EVIDENCE FOR THE LACK OF RESPONSE OF TYROSINE AMINOTRANSFERASE TO DIBUTYRYL CYCLIC AMP IN REGENERATING RAT LIVER

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1. Introduction

Tyrosine aminotransferase (TAT) (EC 2.6.1.5) can be induced in rat liver by glucagon or $N^6,O^{2'}$-dibutyryl cyclic AMP (DBcAMP) [1–3]. The response of the enzyme to DBcAMP, as well as the enzyme itself, is under the control of glucocorticoids and other factors [4]. It is also known that the enzyme in cultured hepatoma cells usually has a lower response to DBcAMP than that in rat liver in vivo [5,6]. Granner [7] has recently reported that TAT in HTC cells, otherwise entirely insensitive to DBcAMP, becomes responsive to the inducer after elevating TAT level by the treatment of cells with dexamethasone (Dex). We also observed that the low response of TAT in an established rat liver cell line (RLC) to DBcAMP can considerably be restored by Dex at a non-inducing concentration (0.1 nM) [8].

In this report we present the evidences showing that TAT in regenerating liver of adrenalectomized rats after partial hepatectomy totally loses its response to the induction by DBcAMP in vivo as well as in vitro in liver slices and that the addition of a non-inducing amount of glucocorticoids partially restores the response. Furthermore, it was demonstrated that the loss of the response is associated with a marked decrease in the cyclic AMP-binding activity in cytosol.

2. Materials and methods

2.1. *Treatments of animals*

Female Wistar rats weighing 120–150 g were adrenalectomized 5–7 days prior to experiments and maintained on a laboratory chow and physiological saline as previously described [4]. Chow was replaced by a low-protein diet (protein content < 1%) 24 h prior to the experiments. Partial hepatectomy was carried out by the method of Higgins and Anderson [9] with removal of 70% of the liver. Cortisone acetate (Ciba AG, Wehr/Baden) and DBcAMP (Boehringer Mannheim GmbH, Mannheim) were injected intraperitoneally. Fractionation of liver for TAT determination was carried out as previously described [4].

2.2. *In vitro TAT induction in liver slices*

For the preparation of slices livers from 3 adrenalectomized rats before and 18 h after partial hepatectomy were sliced by hand with a razor at room temperature. Approximately 100 mg slices were placed in 3 flasks each containing 6 ml Eagle's basal medium supplemented with 2 mM glutamine and DBcAMP at concentrations indicated. The flasks were incubated at 37°C for 3 h in an atmosphere of 95% O_2 and 5% CO_2 with constant shaking. At the end of incubation the slices were washed 3 times with cold saline solution and homogenized in 3 ml cold buffer in a Potter type homogenizer as previously described [4]. TAT and protein were assayed on the 10 000 × g supernatants by the method of Diamondstone modified by Granner and Tomkins [10] and Lowry [11], respectively.

2.3. *Assay for cyclic AMP binding activity*

Cytosols were prepared by homogenizing the livers in 0.02 M potassium phosphate buffer, pH 7.4, containing 0.15 M KCl in a Potter type homogenizer followed by centrifugation at 105 000 × g for 90 min. The binding assay was performed on the dialyzed cytosols by the method of Walton and Garren [12]

in the presence of 5.2×10^{-8} M cyclic [^{3}H]AMP (The Radiochemical Centre, Amersham).

3. Results and discussion

The in vivo results presented in fig.1 show that DBcAMP enhances TAT activity 200% over the saline-treated control level in intact (not adrenalectomized) rats in 3 h (A-1). Partial hepatectomy itself results in a striking increase in the activity probably due to an elevated plasma glucocorticoid level caused by the surgical stress [13] (A-2). Administration of DBcAMP to the intact rats after partial hepatectomy exerts no additional effect on the enzyme activity. In contrast, DBcAMP elevates TAT level only 60% in the adrenalectomized rats is consistent with the previous report [4] (B-1). After partial hepatectomy TAT in the liver of adrenalectomized rats becomes entirely insensitive to the DBcAMP induction, although the basal enzyme level remains low (B-2). Administration of a non-inducing dose of cortisone acetate (0.2 mg/100 g) [4] together with DBcAMP markedly restores the response of TAT to the nucleotide inducer in the regenerating liver of adrenalectomized rats. The loss of the response of TAT to DBcAMP in regenerating liver was also confirmed in an in vitro system using liver slices. As seen in fig.2 TAT can readily be induced by DBcAMP in the non-regenerating liver slices from adrenalectomized rats (curve I). This increment of the TAT activity may represent the glucocorticoid-independent fraction of the enzyme induction [4]. In marked contrast, TAT cannot be induced at all by DBcAMP in the slices from regenerating liver of adrenalectomized rats 18 h after partial hepatectomy (curve II). Addition of a non-inducing concentration of Dex (1 nM) to the medium together with DBcAMP restores the response of the enzyme over the level of non-regenerating liver slices in a range of low DBcAMP concentrations (curve III). This restored response may represent the glucocorticoid-dependent induction medium by DBcAMP. At higher DBcAMP concentrations (> 1.0 mM) Dex is rather inhibitory.

Rijn et al. [6] found no direct correlation between

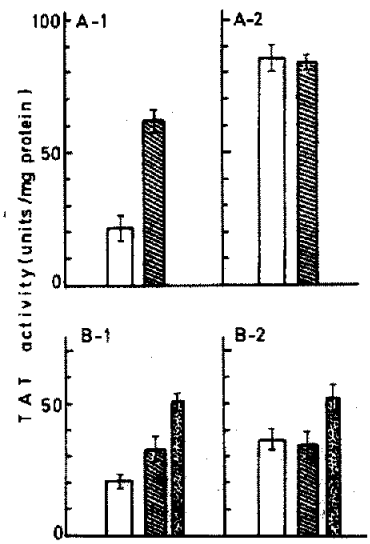

Fig.1. Response in vivo of TAT to DBcAMP-induction in regenerating liver of intact and adrenalectomized rats. DBcAMP (3 mg/100 g) or cortisone acetate (0.2 mg/100 g) was injected intraperitoneally to the normal (not hepatectomized) (A-1, B-1) and the partially hepatectomized rats 4 h after surgery (A-2, B-2) and the rats were killed 3 h later. (A) Intact rats; (B) adrenalectomized rats. (White bar) Saline; (hatched bar) DBcAMP (dotted bar) DBcAMP and cortisone acetate. Data are means ± SE for 5–6 rats.

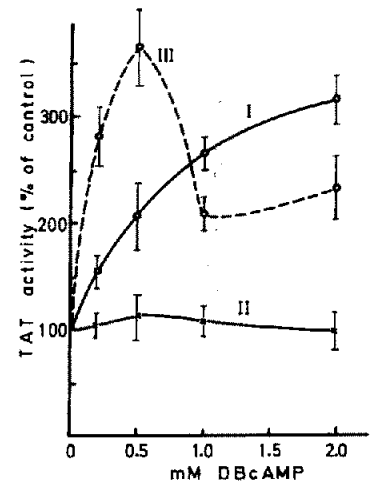

Fig.2. In vitro response of TAT to DBcAMP-induction in the slices from normal and regenerating livers of adrenalectomized rats. Slices prepared from the adrenalectomized rats before (I) and 18 h after hepatectomy (II) were incubated for 3 h with increasing concentrations of DBcAMP and TAT was assayed as described under Materials and methods. Curve III is the same as II except that the slices were incubated in the presence of 1 nM Dex. The data are the means ± SE for 3 incubations.

growth rate and induction of TAT by DBcAMP in four different hepatoma cell lines. The result of Granner [7] also is indicative of the independence of the enzyme response on the growth rate and cell cycle of HTC cells. On the other hand, serine dehydratase was found by Kapp et al. [14] to be inducible by DBcAMP only in the late S-phase in CHO cells. During fetal and neonatal development of rats changes in the response of several hepatic enzymes to glucagon or DBcAMP have been observed [15–18]. In the present study we found the absence of in vivo and in vitro response of TAT to DBcAMP from the cells in the prereplicative phase of the regenerating liver of adrenalectomized rats. Since glucocorticoids do not affect the mitotic activity of regenerating liver [19], our present results suggest that the loss of the glucocorticoid-independent response of TAT to DBcAMP is characteristic of the proliferating cells or cells ready to proliferate. In view of the recent finding that a specific cyclic AMP-binding site is deficient from hepatoma cells [20–23] and that during the developmental period of rat liver the cyclic AMP-binding activity changes [24], the cyclic AMP-binding protein was assayed on the cytosol fractions from the regenerating liver of adrenalectomized rats 18 h after partial hepatectomy. Figure 3 demonstrates a considerable reduction in the cyclic AMP-binding activity of the regenerating liver. Several binding proteins are

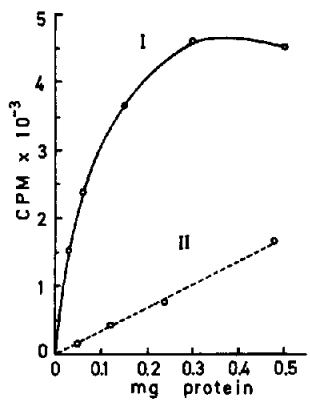

Fig.3. Cyclic AMP-binding activity as a function of protein concentration of cytosols from normal and regenerating livers of adrenalectomized rats. Cytosols were prepared from the livers of adrenalectomized rats before (I) and 18 h after partial hepatectomy (II). Cyclic AMP-binding assay was done on the dialyzed cytosols as described in the text.

reported to be present in rat liver cytosol [22,25] and the lack of one of these proteins was shown with the HTC hepatoma cell line [22], which is highly insensitive to DBcAMP in terms of the TAT induction. It seems likely that the lack of response of TAT to DBcAMP in regenerating liver is associated with the absence of a certain cyclic AMP-binding protein which is essential for the transmission of the cyclic AMP effect.

Acknowledgements

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References

[1] Holten, D. and Kenney, F. T. (1967) J. Biol. Chem. 242, 1372–1377.
[2] Hager, C. B. and Kenney, F. T. (1968) J. Biol. Chem. 243, 3296–3300.
[3] Wicks, W. D., Kenney, F. T. and Lee, K.-L. (1969) J. Biol. Chem. 244, 6008–6013.
[4] Hoshino, J., Kühne, U., Filjak, B. and Kröger, H. (1975) FEBS Lett. 56, 62–65.
[5] Stellwagen, R. H. (1972) Biochem. Biophys. Res. Commun. 47, 1144–1150.
[6] Rijn, H. V., Bevers, M. M., Wijk, R. V. and Wicks, W. D. (1974) J. Cell. Biol. 60, 181–191.
[7] Granner, D. K. (1976) Nature 259, 572–573.
[8] Hoshino, J., Kühne, U., Filjak, B. and Kröger, H. (1976) Hoppe-Seyler's Z. Physiol. Chem. 357, 264.
[9] Higgins, G. M. and Anderson, R. M. (1931) Arch. Pathol. 72, 186–202.
[10] Granner, D. K. and Tomkins, G. M. (1970) in: Methods in Enzymology (Tabor, H. and Tabor, C. W. eds) Vol. 17A, pp. 633–637, Academic Press.
[11] Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Walton, T. M. and Garren, L. D. (1970) Biochemistry 9, 4223–4229.
[13] Desser-Wiest, L., Zwintz, C. and Weiser, K. (1975) Horm. Metab. Res. 7, 75–77.
[14] Kapp, L. N., Remington, J. A. and Klevecz, R. R. (1973) Biochem. Biophys. Res. Commun. 52, 1206–1212.
[15] Holt, P. G. and Oliver, I. T. (1969) Biochemistry 8, 1429–1437.
[16] Young, D. and Oliver, I. T. (1971) Comp. Biochem. Physiol. 40A, 134–144.
[17] Snell, K. and Walker, D. G. (1974) Biochem. J. 144, 519–531.

[18] Heinonen, K. (1975) Biochim. Biophys. Acta 399, 311–323.
[19] Seidman, I., Teebor, G. W. and Becker, F. F. (1967) Cancer Research 27, 1620–1625.
[20] Granner, D. K. (1972) Biochem. Biophys. Res. Commun. 46, 1516–1522.
[21] Granner, D. K. (1974) Arch. Biochem. Biophys. 165, 359–368.
[22] Mackenzie, III, C. W. and Stellwagen, R. H. (1974) J. Biol. Chem. 249, 5755–5762.
[23] Goldberg, M. L., Burke, G. C. and Morris, H. P. (1975) Biochem. Biophys. Res. Commun. 62, 320–327.
[24] Lee, P. C. and Jungmann, R. A. (1975) Biochim. Biophys. Acta 399, 265–276.
[25] Filler, R. and Litwack, G. (1973) Biochem. Biophys. Res. Commun. 52, 159–167.