BINDING OF [^{3}H]DEXAMETHASONE TO RAT LIVER CYTOSOL PROTEINS DURING POSTNATAL DEVELOPMENT

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Received 25 August 1977

1. Introduction

Dexamethasone-binding proteins have been detected in rat liver [1–5]. Many attempts have been made to establish the role of these binding proteins in glucocorticoid-induced functions, such as increased RNA synthesis [6] and enzyme induction [2,7–9]. The fact that the induction of certain liver enzymes is also dependent on the age of the animal [2,7,10–15] led to studies correlating receptor concentrations to the ability to react to exogenously applied hormone. The finding that four dexamethasone-binding components can be demonstrated in rat liver cytosol [5,16] has tempted us to re-examine this problem and to look for a correlation between the developmental appearance of these different forms of receptors and the appearance and inducibility of two liver enzymes, tryptophane oxygenase (TO) and tyrosine aminotransferase (TAT), which are known to be induced by glucocorticoids.

2. Materials and methods

2.1. *Chemicals*

DE-32 cellulose was obtained from Whatman, Heidelberg, FRG; [^{3}H]dexamethasone (spec. act. 25–30 Ci/mmol) from Amersham-Buchler, Braunschweig, FRG; dexamethasone from Sigma, St Louis, MO. All other chemicals were of analytical grade obtained either from Merck, Darmstadt, or from Serva, Heidelberg, FRG.

2.2. *Binding assays*

In the in vivo binding studies 2 μg [^{3}H]dexamethasone/100 g body wt, dissolved in 0.9% NaCl were injected i.p. 25 min before sacrifice. Cytosol was then prepared and the amount of bound radioactivity determined by the charcoal–dextran assay as previously described [5]. In the in vitro experiments various concentrations of [^{3}H]dexamethasone were incubated overnight at 0–4°C with cytosol, then treated with charcoal–dextran and the data evaluated according to Scatchard [17].

2.3. *Determination of enzyme activity*

TAT activity was assayed according to a slightly modified Diamondstone method [18], and TO-activity according to Schütz and Feigelson [19].

2.4. *DEAE-cellulose chromatography*

Performed as described [5].

3. Results

3.1. *Quantitation of total dexamethasone-binding activity of cytosol during postnatal development*

We have quantitated the total dexamethasone-binding activity of rat liver cytosol, starting from animals of two days before birth up to four weeks post partum. Animals of approximately the same body weight stemming from the same litter were used. In a first series, binding capacity was measured after in vitro incubation of the cytosol with dexamethasone.

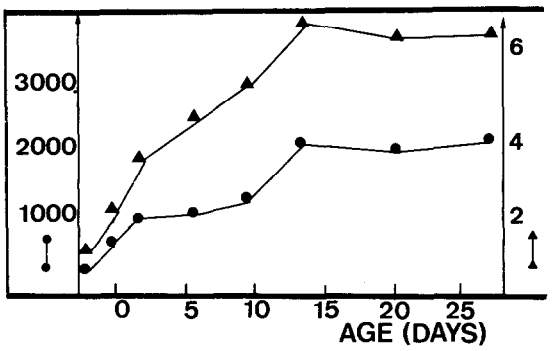

Fig.1. Binding capacity of liver cytosol during the postnatal development of the rat. The binding capacity was measured either in vitro (•——•) or in vivo (▲——▲) (see Materials and methods). The results are expressed either as 10^{-13} mol hormone bound/mg protein for the in vitro, and dpm bound [^{3}H]dexamethasone/mg protein for the in vivo experiments.

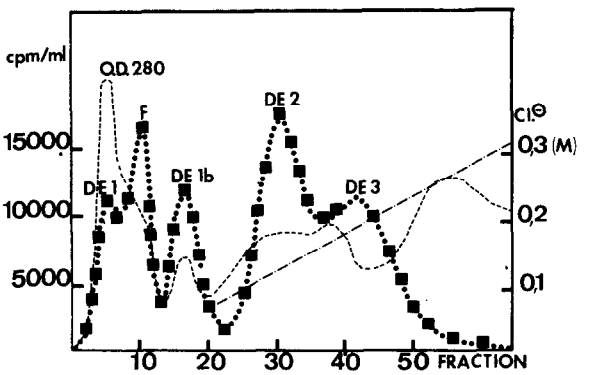

Fig.2. DEAE-cellulose chromatography of rat liver prepared from animals injected i.p. with 2 μg [^{3}H]dexamethasone. (F) free dexamethasone. (– – – –) A_{280}. (·—·—·—) mM KCl. (■ · · ■) [^{3}H]dexamethasone bound/ml.

By use of Scatchard plots [17] both the K_a as well as the binding capacity were determined. In a second series, [^{3}H]dexamethasone was administered intraperitoneally at a dose of 2 μg/100 g body wt, and the bound activity in the cytosol was evaluated. The injected dose of dexamethasone is sufficient to induce maximally both TAT and TO activity (Grote, Voigt, Schmid and Sekeris, manuscript in preparation). As fig.1 shows, the binding capacity of the cytosol is very low in 20 day old embryos, increases at birth and continues to do so up to the end of the second week, then reaching a plateau. The form of the curve is similar in both types of assay, the relative increase of receptor concentration being approx. 6–7-fold in both cases.

3.2. *Quantitation of the different dexamethasone binders during postnatal development*

We then proceeded to quantitate the individual binding proteins during postnatal development. As previously described, four protein fractions binding dexamethasone have been separated on DE-cellulose chromatography [5,16]. This is evident from fig.2 depicting the results of DE-cellulose chromatography of cytosol labelled with [^{3}H]dexamethasone in vivo. Four fractions containing bound dexamethasone are separated: DE-1, in the flow-through fraction; DE-1b, eluting from the column after prolonged washing; and DE-2 and DE-3, adsorbing to the column and eluting at 110 mM and 180–220 mM KCl, respectively. The developmental changes in the concentrations of these different dexamethasone binders after in vivo administration of hormone are depicted in fig.3. DE-1 is present in higher concentrations than the other binders at birth and then increases rapidly, reaching its maximal concentration after five days. Two weeks thereafter, the amount of binder DE-1 slowly decreases. DE-1b is present at birth and its concentra-

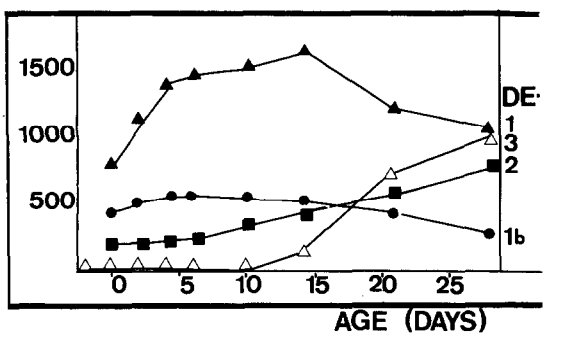

Fig.3. Postnatal fluctuation of the concentration of the four dexamethasone-binding proteins of rat liver cytosol after in vivo administration of the hormone. [^{3}H]Dexamethasone was applied i.p. to rats at different time periods during their postnatal development. Cytosol submitted to DE-32 chromatography. The amount of radioactivity in the individual peaks was determined and expressed as dpm/mg protein. (▲——▲) DE-1. (•——•) DE-1b. (■——■) DE-2. (△——△) DE-3.

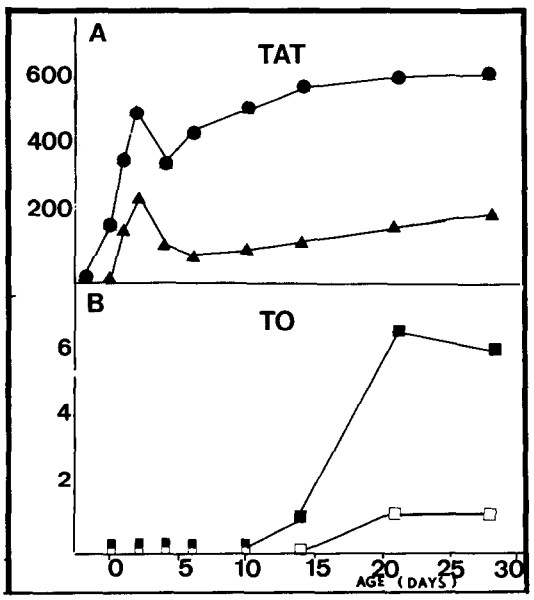

Fig.4. Endogenous and inducible liver activity during postnatal development of rats of TAT and TO. The endogenous TAT activity as well as the activity induced 5 h after the administration of 5 μg/100 g body wt dexamethasone was determined as described in Materials and methods. Fig.4A. (▲——▲) endogenous TAT activity, (●——●)dexamethasone-induced TAT activity. Fig.4B. (□——□) endogenous TO activity (■——■) dexamethasone-induced activity.

tion shows only small fluctuations. DE-2 shows a constant low concentration during the first five days of postnatal development and then increases constantly up to the fourth week. Most interesting is the curve of binder DE-3, which up to ten days after birth is completely absent from the cytosol. Its concentration then increases slowly, then more rapidly, reaching high values at four weeks.

3.3. *TAT and TO activities and inducibility by dexamethasone during postnatal development*

In a last series of experiments we followed the changes in the activities of TAT and TO during postnatal development as well as their inducibility by dexamethasone. The results are shown in fig.4. TAT (fig.4a) activity is low at birth, shows a transient increase immediately after birth with a first maximum at 2–3 days, then decreases to low levels at 5 days, increasing continuously thereafter up to 4 weeks. The enzyme is inducible postnatally by dexamethasone at all time periods tested, but it cannot be induced in 20 day old embryos. The postnatal development of TO (fig.4b) is completely different, no enzyme activity present up to two weeks after birth. Thereafter TO increases to reach adult levels, within a week. The enzyme is not inducible by dexamethasone during the first two weeks of life, but is maximally inducible in three week old animals.

4. Discussion

Several groups have attempted to quantitate glucocorticoid receptors in rat liver during postnatal development in order to investigate the role of these proteins in hormone action [2,6,7,10,14,15]. All authors agree that glucocorticoid-binding capacity of newborn rats is low and increases during postnatal development. Some authors describe a rapid increase of binding capacity with a maximum 2–4 days after birth [10], others a more gradual increase with a maximum after 10 days [2,14,15] or an increase starting after the fifth postnatal day [6,7]. Although the techniques used to determine the binding capacity are very similar, variations in the buffer system used for the cytosol preparation and in the charcoal treatment may partly account for the great variance in the reported results.

In the present paper we have quantitated the dexamethasone-binding capacity of the cytosol by labelling in vivo as well as in vitro, using an optimized buffer system [5]. Although both methods have drawbacks, they rendered, in principle, similar results. In agreement with previous findings [2,6,10,14,15] we have found a very low concentration of receptors before birth, a gradual increase thereafter and a maximal content at the beginning of the third week.

In contrast to the well known gradual increase of the overall binding capacity, the different forms of glucocorticoid receptors, which can be detected after DE-cellulose chromatography, show major fluctuations. In newborn rats, DE-1 and DE-1b were present in the cytosol in concentrations comparable to those present in 4 week old animals, which are fully inducible by glucocorticoids. DE-2, although present in the newborn, continously increases after the first week up to the end of the fourth week. Most interestingly, DE-3

was not detectable during the first ten days after birth, appearing at the end of the second week and then steeply increasing. The correlation between the developmental appearance of DE-3 on the one side and the appearance of TO and its inducibility by dexamethasone on the other side is quite striking. It should be mentioned that other liver enzymes, such as the alanine transaminase [7], show a similar behaviour as TO and are likely candidates for a regulation by the way of DE-3. The demonstration of correlations should not, at the present state of our knowledge, be overestimated; they suggest, however, the involvement of DE-3 in the induction of specific enzymes.

Recent results [20] show that all four dexamethasone-binding proteins share common antigenic sites. In fact, DE-2 and DE-3 are composed of the same 42 000 dalton subunit, which in DE-3 exists as a dimer, very probably covalently linked through disulfide bonds [20]. Many possibilities may, therefore, be discussed relating to the mechanisms of the developmental appearance of the various binders, e.g., the transformation of one receptor form to another by secondary modifications. Work in this direction is currently in progress.

References

[1] Raspé, G. (ed) (1971) Advances in the Biosciences, Vol. 7, Pergamon Press/Vieweg Verlag, Oxford, Braunschweig.
[2] Cake, M. H. and Litwack, G. (1975) in: The Biochemical Action of Hormones (Litwack, G. ed) Vol. III, pp. 317–390, Academic Press, New York.
[3] Beato, M. and Feigelson, P. (1972) J. Biol. Chem. 247, 7890–7896.
[4] Litwack, G., Filler, R., Rosenfeld, S. A., Lichtash, M., Wishman, C. A. and Singer, S. (1973) J. Biol. Chem. 248, 7481–7486.
[5] Schmid, W., Grote, H. and Sekeris, C. E. (1976) Mol. Cell. Endocrinol. 5, 223–241.
[6] Van der Meulen, N. and Sekeris, C. E. (1973) FEBS Lett. 33, 184–186.
[7] Van der Meulen, N., Lipp, K. and Sekeris, C. E. (1974) Klin. Wschr. 52, 571–574.
[8] Holt, P. G. and Oliver, I. T. (1968) Biochem. J. 108, 333–338.
[9] Beato, M., Kalimi, M. and Feigelson, P. (1972) Biochem. Biophys. Res. Commun. 47, 1464–1472.
[10] Singer, S. and Litwack, G. (1971) Endocrinol. 88, 1448–1455.
[11] Franz, J. M. and Knox, W. E. (1967) Biochemistry 6, 3464–3471.
[12] Sereni, F., Kenney, F. T. and Kretchmer, N. (1959) J. Biol. Chem. 234, 609–612.
[13] Greengard, O. (1969) Biochem. J. 115, 19–24.
[14] Cake, M. H., Ghisalberti, A. V. and Oliver, I. T. (1973) Biochem. Biophys. Res. Commun. 54, 983–990.
[15] Henning, S. J., Ballard, P. L. and Kretchmer, N. (1975) J. Biol. Chem. 250, 2073–2079.
[16] Schmid, W. and Grote, H. (1977) in: The Multiplicity of Steroid Hormone Receptors (Agarwal, M. ed) Elsevier, Amsterdam, in press.
[17] Scatchard, G. (1949) Ann. NY Acad. Sci. 51, 660–672.
[18] Diamondstone, T. J. (1966) Anal. Biochem. 16, 395–401.
[19] Schütz, G. and Feigelson, P. (1972) Anal. Biochem. 46, 149–155.
[20] Govindan, M. V. and Sekeris, C. E. (1977) submitted.