CYTOPLASMIC BINDING OF DEXAMETHASONE AND INDUCTION OF TYROSINE AMINOTRANSFERASE IN NEONATAL RAT LIVER

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SUMMARY: Dexamethasone administration markedly increases the activity of tyrosine aminotransferase in postnatal rat liver. The glucocorticoid fails to induce the enzyme in foetal rats when administered *in utero*. Dexamethasone binding activity of rat liver cytoplasm is low or absent in foetal animals but increases to adult levels 1-2 days after birth. *In vitro* experiments with isolated nuclei indicate that foetal nuclei have the capacity to accumulate dexamethasone but only when presented with cytosol-bound glucocorticoid.

INTRODUCTION: In attempts to elucidate the mechanism by which glucocorticoids regulate specific enzyme synthesis in the liver, many investigations have been made of the early interactions of glucocorticoids with the various cellular components. It has been shown that in rat liver there is more than one species of protein capable of binding glucocorticoids with high affinity (1) and it is difficult to determine which of these corresponds to the physiologically effective glucocorticoid receptor. Recently, Beato and Feigelson (2) have shown that rat liver cytosol contains three glucocorticoid binding proteins but only one of these (the G-protein) has high affinity for natural, as well as synthetic, glucocorticoids. Furthermore, when hydrocortisone was administered to adrenalectomized adult rats the amount of hydrocortisone bound to the G-protein *in vivo* at different times and dose levels was found to correlate closely with the extent of hormonal induction of hepatic tyrosine aminotransferase (3). These facts led the authors to suggest the G-protein as the hepatic glucocorticoid receptor.

The present paper establishes that a relationship exists between the presence of dexamethasone binding activity in the cytosol and the induction of hepatic tyrosine aminotransferase by dexamethasone (a synthetic glucocorticoid) in the neonatal rat.

Evidence is presented which supports the hypothesis that the dexamethasone binding protein of rat liver is the glucocorticoid receptor. Furthermore, it seems likely that the reason for the failure of glucocorticoids to induce tyrosine aminotransferase in foetal rats is either that the receptor is absent from the foetal liver, or that endogenous steroids inhibit its activity by masking it.

MATERIALS AND METHODS

Materials: [1,2 - ^{3}H] Dexamethasone (specific radioactivity 22 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Nonradioactive dexamethasone was a gift of Merck, Sharp and Dohme (Aust.) Pty. Ltd.

Injection of animals: Rats of the Wistar albino strain of *Rattus norvegicus* were injected intraperitoneally with 5 µl/g body weight of a dexamethasone suspension (6 mg/ml 0.145 M NaCl), or with an equivalent amount of 0.145 M NaCl. Eight hours later they were killed by cervical fracture and the livers dissected out and weighed. Intraperitoneal injections into foetal animals *in utero* were made through the uterine wall as previously described (4). A 25 µl sample of the above dexamethasone suspension or an equivalent volume of 0.145 M NaCl was injected.

Assay of tyrosine aminotransferase: Livers were washed in cold 0.145 M NaCl, blotted dry, weighed and homogenized in ice-cold 0.25 M sucrose. The homogenate was centrifuged at 25,000 g for 25 min. at 4^{o} in an International PR-6 centrifuge. Tyrosine aminotransferase was assayed in an aliquot of the supernatant by a modification of the method described by Diamondstone (5). The reaction mixture contained 6 µmoles of tyrosine, 0.12 µmoles pyridoxal phosphate, and 26.8 µmoles α-ketoglutarate, in a total volume of 2.2 ml at pH 7.4. Activity is expressed as nmoles p-hydroxyphenyl-pyruvate formed per min per mg protein at 37^{o}.

Measurement of cytosol binding of dexamethasone: Livers were washed in ice-cold saline, dried before weighing and homogenized in 2 volumes of ice-cold medium composed of 0.25 M sucrose, 25 mM KCl, 10 mM $MgCl_2$ and 1 mM mercapto-ethanol in 50 mM Tris-HCl, pH 7.55. The homogenate was centrifuged at 405,000

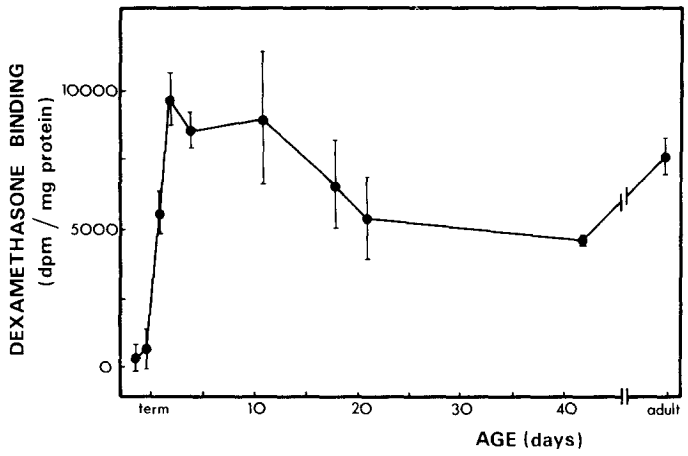

Fig. 1: Dexamethasone binding capacity of postnatal rat liver.

Each point for animals up to 21 days of age represents the mean of determinations on pooled samples from each of 4 different litters. Foetal results were obtained using animals close to term. The points for 42 day old and adult rats represent the means of determinations on 4 male animals from different litters. The vertical bars represent ± 1 S.E. of the mean.

g for 16 minutes at 0-4° in a Spinco L2-65 ultracentrifuge (SW 56 rotor). After discarding the upper lipid layer, the supernatant (cytosol) was immediately assayed using the charcoal absorption technique (3) with an incubation time of 90 min at 0°. To measure the amount of protein bound steroid after charcoal treatment, 200 µl of the supernatant was placed in 10 ml Diotol (6) and the radioactivity determined in a Nuclear Chicago Isocap 300 liquid scintillation counter with an efficiency of 39%. Correction for quenching was based on the channels ratio method. Results are expressed as dpm specifically bound per mg protein.

Cell-free nuclear binding of dexamethasone: Pooled livers from foetal or 2 day old rats were homogenized in 2 volumes of buffered 0.25 M sucrose (containing 25 mM KCl, 10 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM dithiothreitol in 50 mM Tris-HCl, pH 7.55) and the nuclei isolated by the method of Oliver and Blumer (7). The nuclei were finally resuspended in a volume of buffered 0.25 M sucrose such that nuclei from 1 g of liver were taken up in 1.0 ml. By light microscopy, the nuclear fraction was seen to contain hepatocyte nuclei contaminated with some

TABLE 1: Dexamethasone induction of tyrosine aminotransferase in rat liver.

Age	Tyrosine aminotransferase activity (nmoles product formed/min/mg protein) Control	Test
Foetal	1.7 ± 0.3	2.3 ± 0.5
1 day old	83 ± 12	218 ± 10**
2 day old	90 ± 33	305 ± 58**
4 day old	67 ± 52	268 ± 20*
11 day old	140 ± 30	597 ± 88**
42 day old	128 ± 10	352 ± 37**
Adult	78 ± 12	378 ± 67**

Animals of the ages indicated were injected with 3 mg dexamethasone/100 g body weight (test) or an equivalent volume of 0.145 M NaCl (control). After 8 hours exposure the animals were killed and enzyme activity determined. The values represent the mean ± 1 S.E. of 4 experiments. Values of P are denoted by asterisks: * < 0.05, ** < 0.01 (values compared with corresponding control value).

haemopoietic cells. During preparation of the nuclear fraction, additional litters of foetal and 2 day old rats were used for preparation of a cytosol fraction by homogenization of the livers in 1.5 volumes of homogenization buffer. The cytosol was then incubated with 33 nM ^{3}H-dexamethasone for 3 hr at 0^{o} to allow saturation of the specific glucocorticoid binding protein. Mixtures containing 0.4 ml portions of this cytosol were then mixed with one volume of nuclear suspension and transferred to a bath at 20^{o}. After 1 hr the samples were chilled in an ice bath for 10 min and centrifuged for 10 min at 3,000 g. The nuclear pellet was washed twice with 1 ml of buffered 0.25 M sucrose and finally suspended in water. Aliquots were assayed for radioactivity and protein. For determination of the amount of non-specific binding, parallel incubations

TABLE 2: Specific binding of dexamethasone by foetal and 2 day old rat liver nuclei in a cell-free system.

Composition of the Incubation Mixture			
Nuclei (tissue source)	^{3}H-dexamethasone in:	Cytoplasmic-bound ^{3}H-dexamethasone (dpm/mg protein)	Nuclear-bound ^{3}H-dexamethasone (dpm/mg protein)
Foetal liver	Buffer	0	84 ± 300
	Foetal cytosol	616 ± 267	1,575 ± 61
	2-day old cytosol	17,290 ± 1,802	17,000 ± 888
2-day old liver	Buffer	0	2,779 ± 1,038
	Foetal cytosol	616 ± 267	2,832 ± 2,010
	2-day old cytosol	17,290 ± 1,802	13,084 ± 2,606

After incubation of the cytosol fractions or buffer at 0°C for 3 hours with ^{3}H-dexamethasone the specifically bound steroid was determined. An aliquot of each fraction was mixed with an equal volume of nuclear suspension from livers of foetal or 2 day old rats and after incubation at 20°C for 1 hour the specific ^{3}H-dexamethasone binding by nuclei was determined. The values represent the mean ± 1 S.E. of 3 experiments.

were done with cytosol equilibrated with 33 nM ^{3}H-dexamethasone plus a 5,000 fold excess of non-radioactive dexamethasone as a competitor. Specific binding equals the total minus the non-specifically bound steroid.

RESULTS AND DISCUSSION: The activity of tyrosine aminotransferase is known to be low in foetal rat liver (8,9). This is confirmed by the results presented in Table 1. Furthermore, it can be seen that dexamethasone fails to cause an increase in tyrosine aminotransferase activity in foetal rats but causes marked increases in this enzyme when administered to postnatal animals. The exposure time to dexamethasone was chosen as 8 hours as this is the time of optimal effect of the glucocorticoid in both 2 day old and adult rats. Even after a

prolonged exposure of 15 hours, dexamethasone failed to induce tyrosine aminotransferase in foetal rats *in utero*.

Inconsistent findings have been reported on the effect of glucocorticoids on hepatic tyrosine aminotransferase of postnatal rats. Singer and Litwack (10) reported that up to 11 days after birth hepatic tyrosine aminotransferase levels are not affected by hydrocortisone administration. However, Franz and Knox (11) demonstrated marked increases in tyrosine aminotransferase in response to administration of the steroid from the age of 2 days. Other workers (8,12) have demonstrated marked increases in the enzyme activity of 2 day old rats after injection of hydrocortisone. Corticosterone has also been shown to increase enzyme activity postnatally but has no effect in foetal rats (unpublished observations).

Liver contains several glucocorticoid binding proteins (1) and this has complicated studies on the mechanism of action of these hormones. However, Beato and Feigelson (2) have shown that only one of these binds the biologically active synthetic glucocorticoid, dexamethasone, and concluded that this protein is the intracellular receptor for glucocorticoids. The dexamethasone binding capacity of rat liver cytosol as a function of age is shown in Fig. 1. The results show that there is very low binding activity in foetal rat liver, but the activity increases postnatally, reaching adult levels 1-2 days after birth.

The hypothesis that the dexamethasone binding protein is the glucocorticoid receptor is supported by the findings that foetal rat lacks the ability to bind dexamethasone and is unresponsive to the hormone, whereas postnatal animals have dexamethasone binding activity and respond to the hormone. The appearance of dexamethasone binding activity in postnatal animals might thus account for the postnatal development of tyrosine aminotransferase. Evidence of decreased cytoplasmic binding in other cases of diminished response to hormones has been reported (13-16). On the other hand, Shymala (17) has shown in estradiol-independent mouse mammary tumours that, although the cells contain the cytoplasmic receptor, the hormone fails to accumulate in the nucleus.

In this investigation experiments with liver nuclei from foetal and 2 day old rats (Table 2) establish that the hormone accumulates in the nuclei when incubated with the steroid in the presence of 2 day old rat liver cytosol. This implies that foetal nuclei have no impairment in the mechanism of transfer of the hormone from the cytoplasm to the nucleus, or in the specific nuclear binding sites. The low level of nuclear binding when the nuclei were incubated with steroid in the presence of foetal rat liver cytosol suggests that the inability to concentrate the steroid in the nucleus is a result of the impaired cytoplasmic binding.

Because the binding assay used in this investigation measures only the unbound binding protein (18), we cannot conclude whether the absence of dexamethasone binding activity in foetal rat liver is a result of a lack of the binding protein or a result of saturation of the binding protein with endogenous steroid. This aspect is presently under investigation.

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