STIMULATION OF TYROSINE AMINOTRANSFERASE OF FETAL RAT LIVER BY CORTISOL, 17,21-DIHYDROXY-PREGN-4-EN-3,20-DIONE (11-DEOXYCORTISOL) AND 11-DEOXYCORTICOSTERONE

Synergistic interactions with dibutyryl cyclic AMP

Carl MONDER and Alena Hatle COUFALIK

Research Institute for Skeletomuscular Diseases, Hospital for Joint Diseases and Medical Center, 1919 Madison Avenue, New York, NY 10035, USA

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1. Introduction

There is no evidence to support the idea that cyclic nucleotides are 'second messengers' for steroid action. It has been shown, however, that glucocorticoids, such as cortisol (F) or dexamethasone, in the presence of dibutyryl cyclic AMP (DBCAMP) amplifies liver tyrosine aminotransferase (TAT) activity to a level far greater than that attained by either component alone [1–4].

Here we show that such interactions occur with fragments of normal fetal rat liver in organ culture under conditions where neither nucleotide nor steroid have any effect alone. Furthermore, we show that 11-deoxycortisol (S) and 11-deoxycorticosterone (DOC), steroids generally considered to be incapable of inducing TAT in normal tissue can do so under organ culture conditions.

2. Materials and methods

2.1. *Chemicals*

Cortisol, 11α,17,21-trihydroxy-pregn-4-ene-3,20-dione (11α-cortisol), 11-deoxycorticosterone and 17,21-dihydroxy-pregn-4-ene-3,21-dione (11-deoxycortisol) were bought from Steraloids Corp. (Wilton, NH). All steroids were homogeneous when checked by thin-layer chromatography. Cyclic nucleotides were bought from Calbiochem Corp. (La Jolla, CA).

2.2. *Animals*

Sprague-Dawley rats were purchased from Camm Res. Inst. and maintained at 72°C and 50% humidity. Food and water were supplied ad libitum. The light–dark cycle was 12 h each. Animals, one of each sex per cage, were mated during the 12 h dark period. Fetuses were removed under sterile conditions from pregnant rats anesthetized with ether. Fetal animals at day 20 of gestation were killed by decapitation. The livers were immediately placed in ice-cold 0.14 M KCl.

2.3. *Organ culture*

The procedures for establishing fetal liver explants in organ culture are the same as those we used in [5]. When alcohol solutions of steroids were introduced into the culture vessels, ethanol was 0.05% final conc. Controls with no steroid contained 0.05% ethanol.

2.4. *Enzyme activity*

Liver fragments from one grid were washed in 0.14 M KCl and homogenized at 0°C in 1 ml 0.9% KCl with an axial homogenizer (Teflon in glass). The supernatant fraction obtained after centrifuging at 18 000 × *g* for 15 min was used for estimation of enzyme activity. Tyrosine aminotransferase was measured by the method in [6]. Activity is expressed as μg *p*-hydroxyphenylpyruvate formed. mg protein^{-1} .20 min^{-1}. Proteins were measured in the liver supernatant fluid by the Lowry method [7].

3. Results

The ability of several steroids to stimulate TAT in fetal rat liver explants during short incubation times (i e , for 6 h after establishing explant) is shown in table 1 The results listed in the 'no supplement' column confirm that cortisol does not increase activity in this interval 11-deoxycortisol, DOC, tetrahydrocortisol (THF) and the 11α-epimer of cortisol (11α-F) were also inactive When DBCAMP was introduced at a level which itself produced no increased TAT, a rise in enzyme activity occurred with F, S and DOC Neither THF nor the 11α-F had any effect

Stimulation of TAT in explants by corticosteroids requires that the fragments be first subjected to a preliminary incubation period of 24–48 h in the absence of steroid [1] Under these conditions, F (2 μM) increased TAT ~3-fold in 6 h (fig 1) To our surprise, S at 2 μM also increased TAT about the same amount DOC, though less active, was still effective THF and 11α-F did not stimulate TAT at all When the steroids were tested at 2 nM, none was effective

The addition of DBCAMP to preincubated cultures greatly magnified the effects of the steroids The relative increases were the same as in the absence of nucleotide DBCAMP itself increased TAT ~4-fold THF and 11α-F were again inactive, and did not decrease induction by DBCAMP At 20 nM, F was still a strong inducer and activities of DOC and S were reduced, but measureable

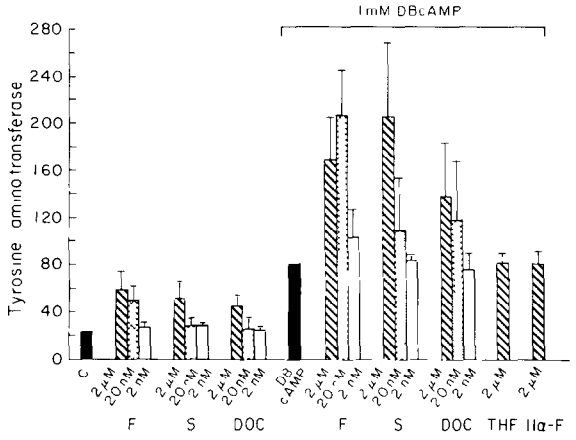

Fig 1 Stimulation of tyrosine aminotransferase activity by steroids and mixtures of steroids with dibutyryl cyclic AMP Bars indicate means, limit lines indicate standard deviations Values beneath bars show molarity of steroid in the culture dishes DBC AMP was present at 1 mM final conc Cultures were preincubated for 42 h, then additions were made for an additional 6 h F, cortisol, S, 11-deoxycortisol, DOC, 11-deoxycorticosterone, THF, tetrahydrocortisol 11α F, 11α-hydroxy epimer of cortisol, C, control Activity is μg *p*-hydroxyphenyl pyruvic acid formed mg protein^{-1} 20 min^{-1}

The ability of mixtures of DBCAMP and steroid to stimulate TAT was probably due to net enzyme synthesis Addition of cycloheximide to the culture medium completely blocked the inducers

Although F, S or DOC added to cultures after 42 h preincubation increased TAT over the following 6 h,

Table 1
Effect of steroids on the increase in tyrosine aminotransferase activity of fetal rat liver explants with no preincubation[a]

Steroid	Enzyme act (μg *p*-hydroxyphenyl pyruvate mg protein^{-1} 20 min^{-1})	
	No supplement	With DBC AMP
None	2 3 ± 0 9	3 4 ± 1 5
Cortisol	4 2 ± 2 2	13 1 ± 3 3
11-deoxycortisol	1 2 ± 0 1	7 3 ± 1 1
11-deoxycorticosterone	1 8 ± 1 5	9 8 ± 2 9
tetrahydrocortisol	2 8 ± 0 4	4 5 ± 1 6
11α-Cortisol	2 0 ± 0 2	4 3 ± 0 7

[a] Final concentration of DBC AMP was 1 mM and steroids were 2 μM

Incubation interval was 6 h after addition of the components indicated There was no preliminary incubation period Values are means and SD for 4–6 experiments

Table 2
Tyrosine aminotransferase activity of fetal rat liver explants incubated continuously with steroids for 48 h

Steroid	Enzyme activity[a]	
	No supplement	with DBC AMP
none	22.9 ± 3.0	80.1 ± 20.4
cortisol	142.6 ± 56.4	200.0 ± 67.9
11-deoxycortisol	30.0 ± 5.9	56.8 ± 16.8
11-deoxycorticosterone	23.9 ± 6.6	75.6 ± 26.8

[a] Dibutyryl cyclic AMP was 1 mM and steroids were 2 μM

Values are means and S.D. of 6–10 replicates

continuous incubation of the steroids with explants for the entire 48 h interval led to increased TAT only with F (table 2). The inability of S and DOC to induce TAT persisted even if DBCAMP was present during the entire incubation period. For cortisol, the net increase in TAT was not greater than the sum of the individual effects of steroid and cyclic nucleotide under these conditions.

4. Discussion

It has long been known that S and DOC are devoid of systemic glucocorticoid activity in experimental animals and humans [8–15]. That these steroids could stimulate TAT activity in fetal liver is therefore unexpected. The concentrations which induced TAT in the presence of DBCAMP were 2×10^{-8} M, well within the physiological range. In the absence of nucleotide, higher levels of steroid were needed to induce the enzyme, but the effects were unmistakeable. With or without nucleotide, maximum induction with S was as great as with cortisol. Evidently, the 11β-hydroxy group, generally accepted as an obligatory structural feature of the glucocorticoids, is not always required for activity. The effects on normal fetal liver of the steroids studied here do not parallel those on HTC cells [16,17].

The synergistic effect of corticosteroids and DBCAMP on induction of TAT is well known [1–5], though the mechanism of interaction is not clear. Most workers find that the mixed components amplify an induction initiated by either one alone. In only one instance prior to our report was there no response to either component alone, and an obligatory need for both steroid and nucleotide to initiate new TAT production [18]. These results and ours contrast with those in [19] where it was found that with hepatoma cells in tissue culture, concentrations of dexamethasone that do not induce TAT alone fail to support the induction by dibutyryl cyclic AMP.

Why cortisol, S and DOC by themselves show a significant stimulatory effect on TAT in explants only after 42 h preincubation may be related to an increase in unoccupied corticosteroid receptors during this time [20,21]. In addition, the rate at which the freshly isolated explant converts steroids to inactive tetrahydro metabolites is initially rapid. Endogenous and added steroids are rapidly catabolized. Metabolism is slow after preincubation [22], and as a result, the active steroid level is maintained longer. The high rate of steroid catabolism of freshly isolated liver may also explain the inability of S and DOC to induce TAT when incubated with explants for the entire 48 h. Extensive metabolism to tetrahydro metabolites during the early hours of incubation occurs and consequently the final level of active unchanged steroid is too low to be effective.

Acknowledgements

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