EARLY GLUCOCORTICOID-DEPENDENT STIMULATION OF RNA POLYMERASE B IN RAT THYMUS CELLS

Neil M. BORTHWICK and Philip A. BELL
Tenovus Institute for Cancer Research, Welsh National School of Medicine, The Heath, Cardiff, CF4 4XX, UK

Received 9 October 1975

1. Introduction

Previous studies [1,2] on the action of glucocorticoids in lymphoid tissues have shown that various catabolic effects interfere with RNA synthesis either directly or indirectly. A decrease in DNA-dependent RNA polymerase (nucleoside triphosphate-RNA nucleotidyltransferase: EC 2.7.7.6) activity in rat thymus cells has been demonstrated in vivo 3 h after the administration of cortisol [3,4]. Although several studies have implicated an early stimulation of RNA in response to the hormone by indirect evidence such as the use of inhibitors of RNA and protein synthesis [5–7], no direct evidence has previously been obtained. This paper describes an early glucocorticoid-dependent increase in RNA polymerase B activity in rat thymus cells within 10 min of steroid administration in vitro.

2. Experimental

2.1. *Materials*

[5-^{3}H]UTP (1 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, Bucks, UK, ATP, CTP, GTP and UTP were supplied by Boehringer (Mannheim, West Germany), α-amanitin was obtained from Calbiochem Ltd., London, and dexamethasone (9α-fluoro-16α-methyl-11β, 17, 21-trihydroxypregna-1,4-diene-3,20-dione) was the product of Sigma (London) Chem. Co. Ltd. Minimal Essential Medium (Eagle): (MEM) was supplied by Gibco Bio-Cult (Paisley, Scotland).

2.2. *Experimental animals*

Male Sprague–Dawley rats weighing 250–350 g at age of 7–8 weeks were adrenalectomised 1 week before use. In in vivo experiments, animals were injected under ether anaesthesia via the jugular vein with dexamethasone (10 μg/100 g body wt) in 0.9% saline. Control animals received vehicle alone.

2.3. *Preparation of thymus cell suspension*

The animals were killed by decapitation, the thymi removed and washed in ice-cold 0.9% NaCl. The tissue was minced and hand-homogenised in a Teflon–glass homogeniser (1 mm clearance) in 20 ml MEM. The homogenate was filtered through 4 layers of muslin and centrifuged for 2 min at 600 *g*. After removal of supernatant, the pellet was resuspended in MEM and recentrifuged for 2 min at 600 *g*. The final pellet was then suspended in MEM, with a final cell concentration of 3–5%.

2.4. *In vitro incubations*

Cell suspensions were incubated at 37°C in a shaking water bath under an atmosphere of 95% O_2/5% CO_2. Dexamethasone was added to a final concentration of 10^{-6} M; control incubations received saline alone.

2.5. *Preparation of nuclei from cell suspensions*

Nuclei were isolated from thymus cell suspensions by the hypo-osmotic shock technique [8]. The suspension was centrifuged for 15 min at 800 *g* and the crude nuclear pellet was resuspended in 0.32 M sucrose. Purified nuclei were prepared by the method of Blobel and Potter [9].

North-Holland Publishing Company Amsterdam

2.6. *RNA polymerase assay*

RNA polymerase activity was assayed in isolated nuclei in either low salt + α-amanitin to measure RNA polymerase A activity or high salt to measure RNA polymerase B activity. The reaction mixture contained in a final volume of 0.08 ml (including 0.05 ml nuclear suspension) 0.075 μmol each of ATP, CTP and GTP, 0.005 μmol of [^{3}H]UTP (0.5 μCi), 0.8 μmol Tris–HCl, pH 8, 0.2 μmol of 2-mercaptoethanol and 5% (v/v) glycerol. Under low-salt conditions, the mixture also contained 0.24 μmol $MgCl_2$, 4 μmol of KCl and 0.1 μg of α-amanitin. Assays carried out in high-salt conditions contained 0.32 μmol $MnCl_2$ and 16 μmol of $(NH_4)_2SO_4$. Assays were started by the addition of 0.05 ml of nuclear suspension to reaction mixture and incubation carried out at 37°C for 15 min. Samples of the reaction mixture were used to determine enzyme activity as described previously [10].

Radioactivity was measured in a Nuclear Chicago Mark II liquid scintillation spectrometer with tritium counting efficiency of 28–32%.

DNA determinations were carried out by the method of Burton [11].

3. Results

3.1. *Effect of dexamethasone on RNA polymerase activity in vivo*

Most previous work [3,4] on the inhibition of RNA polymerase by cortisol in vivo was carried out with a minimum time of 3 h between hormone administration and enzyme assay. In the present experiments, both RNA polymerase A and B activities have been measured from 30 min to 3 h after a single i.v. injection of dexamethasone. Fig.1a shows the activity of RNA polymerase A in isolated thymus nuclei. From 30 min to 1 h after hormone administration, the enzyme activity remains similar to that in control animals but thereafter decreases to about 70% of control values by 3 h. A similar pattern is obtained with RNA polymerase B activity (fig.1b). Again, the enzyme activity remains at control values from 30 min to 1 h and then decreases to 70–75% of control values 3 h after dexamethasone treatment. Since it is not possible to examine time intervals much shorter than 30 min in vivo, further studies were carried out using suspension of thymus cells in vitro.

3.2. *Effect of dexamethasone on RNA polymerase activity in vitro*

Incubation of thymus cell suspensions with 10^{-6} M dexamethasone in vitro facilitates the measurement of RNA polymerase activities in thymus nuclei at much

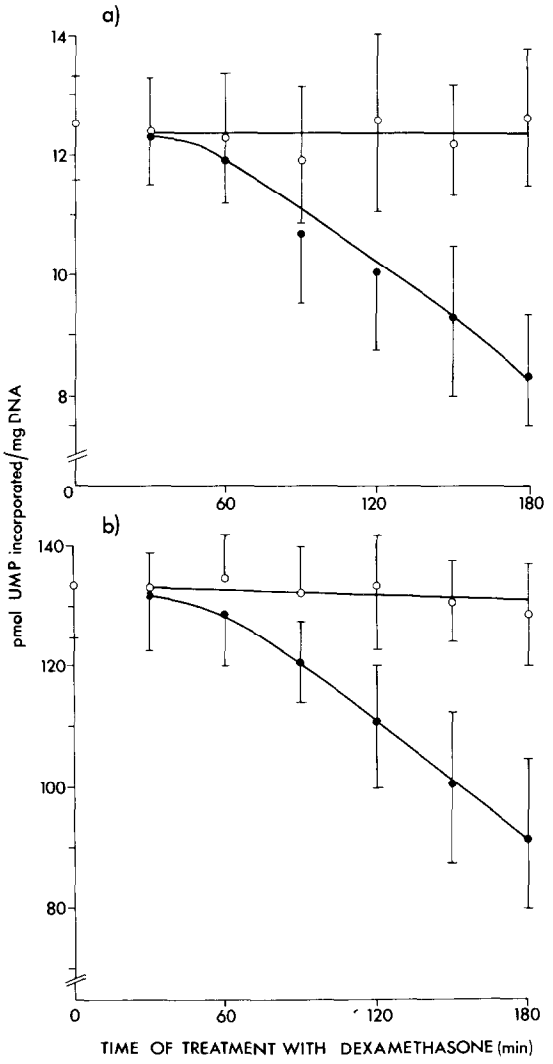

Fig.1. Effect of dexamethasone in vivo on RNA polymerase activities in rat thymus. Rats were treated with dexamethasone for periods up to 3 h and nuclei were prepared from thymi and assayed for RNA polymerase activity as described in the Experimental section. Each point represents the mean ± S.D. obtained from at least six determinations.(a) RNA polymerase A activity in dexamethasone-treated (•) and control (○) thymi. (b) RNA polymerase B activity in dexamethasone-treated (•) and control (○) thymi.

earlier time intervals. RNA polymerase A activity (fig.2a) is identical to the obtained in vivo after hormone treatment although the degree of variation is considerably reduced. The decrease in enzyme activity is evident from 1 h and reaches 30% by 3 h. However steroid treatment produces a striking effect on RNA polymerase B activity in vitro previously undetected in the in vivo experiments (fig.2b). It is apparent that there is a rapid burst of RNA polymerase B activity 10 min after hormone addition. This activity reaches a maximum at 15–20 min at about 15–20% above the corresponding control values before declining to control levels by 30–40 min with the subsequent inhibition of activity after 1 h as already shown in the in vivo experiments.

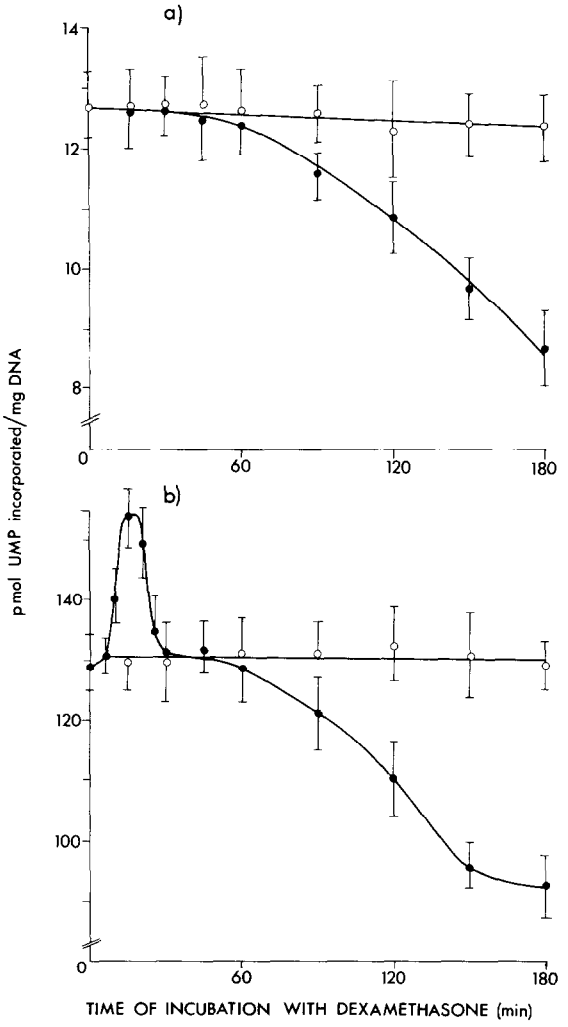

Fig.2. Effect of dexamethasone in vitro on RNA polymerase activities in rat thymus cells. Thymus cell suspensions were incubated with 10^{-6} M dexamethasone for periods up to 3 h and nuclei were prepared and assayed for RNA polymerase activity as described in the Experimental section. Each point represents the mean ± S.D. obtained from six determinations. (a) RNA polymerase A activity in dexamethasone-treated (•) and control (○) thymus cells. (b) RNA polymerase B activity in dexamethasone-treated (•) and control (○) thymus cells.

4. Discussion

The majority of metabolic effects of glucocorticoids on thymic lymphocytes have been shown to be blocked or counteracted by inhibitors of RNA synthesis such as actinomycin D or cordycepin. Examples are the inhibition of the decrease in generation of glucose 6-phosphate from glucose [5,7] and of the decrease in hexose transport [12]. The period of sensitivity to RNA synthesis inhibitors generally precedes the sensitivity to inhibitors of protein synthesis such as cycloheximide. In thymic lymphocytes the inhibitory effects of glucocorticoids on glucose metabolism, which occur 15 min after hormone administration, are blocked only if the inhibitors are present during the first 5–10 min after exposure to hormone. These experiments imply the involvement of an early stimulation in RNA synthesis as an early effect of glucocorticoids on target tissue. In this paper, we have demonstrated that such an early stimulation in RNA synthesis does occur in vitro (fig.2). The enhancement of RNA polymerase B activity in isolated thymic nuclei by 15–20%, 10–15 min after dexamethasone treatment, is the first direct demonstration of the involvement of RNA synthesis in the primary action of this hormone. It is probable that the increase in enzyme activity observed in isolated nuclei is a consequence of changes in the thymic chromatin, arising directly from the interaction of dexamethasone and its receptor protein with specific sites in the chromatin. RNA polymerase B is believed to synthesise high molecular weight nuclear RNA which may be the precursor for mRNA [13]. The increased level of mRNA could result either from an increase in the rate of transcription or a decrease in the rate of degradation; this problem is now under study.

It is believed that some of the inhibitory and

catabolic effects of glucocorticoids are mediated by a decrease in glucose uptake, which leads to decreased incorporation of precursors into RNA and lower ATP levels, although substrates other than glucose can function in its place [14]. The paths of glucocorticoid action may separate early in the response, perhaps through the induction of several different proteins, one of which inhibits glucose uptake and another which initiates the mechanisms resulting in cell death.

Acknowledgement

The authors wish to express their sincere thanks to the Tenovus Organisation for continuing financial support.

References

[1] Fox, K. E. and Gabourel, J. D. (1967) Mol. Pharmacol. 3, 479–486.
[2] Drews, J. and Wagner, L. (1970) Eur. J. Biochem. 13, 231–237.
[3] Nakagawa, S. and White, A. (1970) J. Biol. Chem. 245, 1448–1457.
[4] Makman, M. H., Nakagawa, S., Dvorkin, B. and White, A. (1970) J. Biol. Chem. 245, 2556–2563.
[5] Mosher, K. M., Young, D. A. and Munck, A. (1971) J. Biol. Chem. 246, 654–659.
[6] Hallahan, C., Young, D. A. and Munck, A. (1973) J. Biol. Chem. 248, 2922–2927.
[7] Young, D. A., Barnard, T., Mendelsohn, S. and Giddings, S. (1974) Endocrine Res. Comm. 1, 63–72.
[8] Wira, C. and Munck, A. (1970) J. Biol. Chem. 245, 3436–3438.
[9] Blobel, G. and Potter, V. R. (1966) Science 154, 1662–1665.
[10] Borthwick, N. M. and Smellie, R. M. S. (1975) Biochem. J. 147, 91–101.
[11] Burton, K. (1956) Biochem. J. 62, 315–323.
[12] Makman, M. H., Dvorkin, B. and White, A. (1971) Proc. Nat. Acad. Sci. USA 68, 1269–1273.
[13] Darnell, J. E., Jelinek, W. R. and Molloy, G. R. (1973) Science 181, 1215–1221.
[14] Young, D. A. (1970) J. Biol. Chem. 245, 2747–2752.