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GLUCOCORTICOID EFFECTS ON DNA-DEPENDENT RNA POLYMERASE ACTIVITY IN RAT THYMUS CELLS

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SUMMARY

In vitro incubation of rat thymus cells with the synthetic glucocorticoid dexamethasone resulted in the stimulation of DNA-dependent RNA polymerase B activity within 10 min of steroid addition. This early enhancement of enzyme activity was followed by the inhibition of both RNA polymerase A and B activities, first apparent between 60 and 90 min after steroid addition. When the incubations were performed in media lacking any energy source, the inhibitory effect of dexamethasone on RNA polymerase A activity was abolished, but both the stimulatory and inhibitory effects on RNA polymerase B activity were maintained. The inhibitory effect of dexamethasone on RNA polymerase A also could be abolished by prior treatment with cycloheximide, but not by the addition of cycloheximide 15 min after the steroid. It is concluded that the inhibition of ribosomal RNA synthesis by glucocorticoids is dependent on protein synthesis. Possible relationships between the effects of glucocorticoids on RNA polymerase activities and on the inhibition of DNA synthesis and lymphocytolysis are discussed.

INTRODUCTION

Exposure of rat thymus cells to cortisol *in vitro* results in an inhibition of glucose metabolism, first apparent within 15–20 min after steroid addition [1]. This early response to glucocorticoids is followed at later times by the inhibition of many other metabolic processes including protein synthesis [2], nucleoside transport [3], and DNA-dependent RNA polymerase (nucleoside triphosphate—RNA nucleotidyltransferase: EC 2.7.7.6) activity [4, 5]. We have recently reported that *in vitro* incubation of rat thymus cells with 10^{-6} M dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione) results in the stimulation of RNA polymerase B activity within 10 min of steroid addition [5]. This stimulatory effect is probably one of the earliest actions of the steroid in the nucleus of the thymus cell. In the work reported here, we have investigated the effects of carbohydrate deprivation and inhibition of protein synthesis on the RNA polymerase activities of rat thymus cells for up to 3 h after steroid addition *in vitro*.

EXPERIMENTAL

All procedures for the preparation of thymus cell suspensions, *in vitro* incubations, preparation of purified nuclei, and assay of RNA polymerase activities were carried out as described previously [5]. Dexamethasone was added to a final concentration of 10^{-6} M; control incubations received vehicle alone. Where added, cycloheximide was used at a final concentration of 10^{-4} M and glucose at 1 mg/ml.

RESULTS

Thymus cell suspensions were incubated with 10^{-6} M dexamethasone in Eagles Minimal Essential Medium (MEM) in the presence and absence of glucose. The RNA polymerase activities of nuclei isolated at various times after steroid addition are shown in Fig. 1. In the presence of both dexamethasone and glucose, RNA polymerase A activity was progressively inhibited, by up to 30% at 3 h, while the corresponding activity in the presence of steroid but absence of glucose was inhibited by only about 10% of the control value at 3 h (Fig. 1a). In contrast, RNA polymerase B activity was inhibited by about 25–30% after 3 h in the presence of dexamethasone regardless of whether glucose was present in the incubation medium or not (Fig. 1b). The early transient stimulation of the activity of the B polymerase was likewise unaffected by the presence or absence of glucose.

Since MEM contains amino acids which might possibly act as an alternative energy source, thymus cells were also incubated in Eagles Basal Salt Solution (BSS). RNA polymerase A activity of nuclei from cells incubated in this latter medium was again inhibited by approximately 30% at 3 h when both glucose and dexamethasone were present, but no significant difference in enzyme activity between dexamethasone-treated cells and controls could be observed when glucose was absent (Fig. 2a). RNA polymerase B activity in nuclei from cells incubated in BSS showed a profile similar to that obtained using MEM; the enzyme activity was inhibited to the same degree (25% at 3 h) by dexamethasone in both the presence and absence of glucose. Glucose deprivation by itself

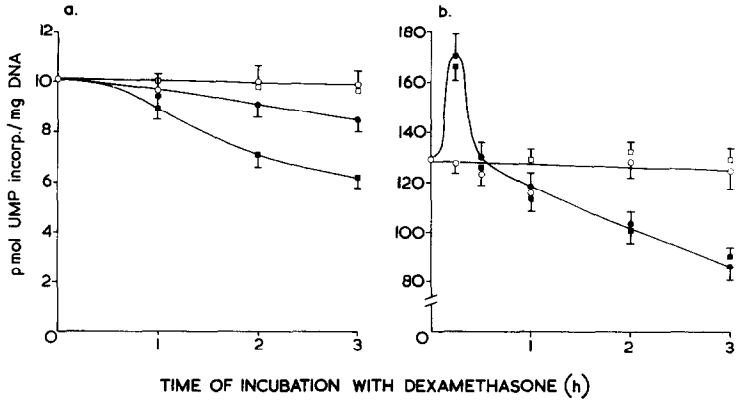

Fig. 1. Effect of dexamethasone on the activity of (a) RNA polymerase A and (b) RNA polymerase B in nuclei from rat thymus cells incubated in MEM. Cells were incubated from zero time with (solid symbols) or without (open symbols) 10^{-6} M dexamethasone in the presence (squares) or absence (circles) of 1 mg/ml glucose. Vertical bars indicate S.D.

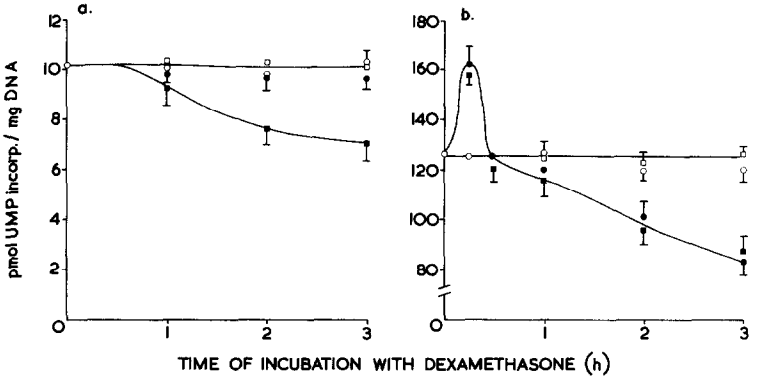

Fig. 2. Effect of dexamethasone on the activity of (a) RNA polymerase A and (b) RNA polymerase B in nuclei from rat thymus cells incubated in BSS. Cells were incubated from zero time with (solid symbols) or without (open symbols) 10^{-6} M dexamethasone in the presence (squares) or absence (circles) of 1 mg/ml glucose. Vertical bars indicate S.D.

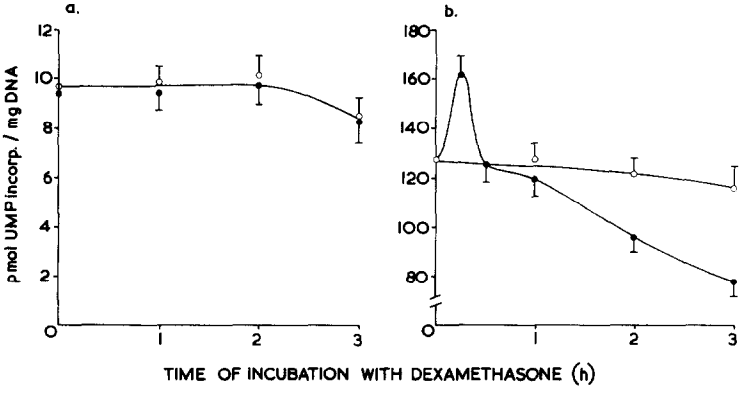

Fig. 3. Effects of cycloheximide and dexamethasone on (a) RNA polymerase A and (b) RNA polymerase B activities in nuclei from rat thymus cells incubated in MEM plus glucose. 10^{-6} M Dexamethasone (solid circles) or vehicle alone (open circles) was added to cell suspensions 15 min after the addition of cycloheximide to 10^{-4} M. Vertical bars indicate S.D.

had no effect on the activity of either enzyme in these experiments.

The effects on RNA polymerase activities of adding cycloheximide to suspensions of thymus cells in MEM plus glucose were studied in experiments in which cycloheximide was added either 15 min before or 15 min after dexamethasone. Addition of the protein synthesis inhibitor 15 min before the steroid resulted in the complete abolition of the steroid effect on RNA polymerase A activity for up to 3 h (Fig. 3a). The same treatment had no effect, however, on either the early glucocorticoid stimulation of RNA polymerase B activity or its subsequent inhibition (Fig. 3b). If the addition of cycloheximide was delayed until 15 min after dexamethasone, when the stimulation of RNA polymerase B is maximal, then the full inhibitory effect of the steroid on the form A polymerase (Fig. 1a) was obtained for the 3 h period studied. RNA polymerase B activity was likewise unaffected by the delayed addition of cycloheximide.

DISCUSSION

This investigation has demonstrated that there are differential effects of both glucose and cycloheximide on the RNA polymerase activities of rat thymus cells following glucocorticoid administration. In the presence of glucose, dexamethasone caused an early enhancement of RNA polymerase B activity which was followed by the inhibition of both RNA polymerase A and B activities, first apparent between 60 and 90 min after steroid addition. When the incubations were performed in glucose-free media, the inhibitory effect of dexamethasone on RNA polymerase A activity was abolished. The slight difference between the results for cells incubated in BSS and MEM is probably due to the ability of amino acids in MEM to act as an alternative energy source in place of glucose. Substrates other than glucose have previously been shown to function in its place [6]. Both the early stimulation and the later inhibition of RNA polymerase B activity by dexamethasone were, however, unaffected by the presence or absence of glucose. A similar differential response of the two polymerase enzymes was also displayed towards cycloheximide when it was added 15 min before the steroid, but delayed addition of the inhibitor was without any effect on the steroid-induced inhibition of RNA polymerase A activity.

Early endocrine effects on carbohydrate metabolism, resulting in an inhibition of carbohydrate-dependent ATP production, have been postulated to be responsible for many of the subsequent biochemical responses of lymphoid cells to glucocorticoids [7]. While this would appear to be the likely explanation of the steroid effects on protein synthesis and nucleoside phosphorylation, processes which are themselves glucose-dependent [3], it is clearly not the explanation of the inhibitory effects on RNA polymerase B, which are independent of carbohydrate metabolism. It is also unlikely that effects on ATP production account for the glucose-dependent inhibition of RNA polymerase A activity since glucose deprivation alone has no effect on the activity of this enzyme. The experiments with cycloheximide suggest instead that this effect is due to the synthesis of a protein factor which inhibits ribosomal RNA synthesis; a search for such a factor is presently being conducted.

It is of interest to speculate on the relationships between the actions of glucocorticoids on RNA polymerase activity and the ultimate cellular responses to these steroids, which in rodent lymphocytes are both cytostatic and cytotoxic in nature. The cortisol-induced lymphocytolysis of P1798 tumour cells is glucose-independent [8], as is what may be a closely related effect, the increased 'nuclear fragility' of rat thymus cells in response to glucocorticoids [9]. The latter effect has also been shown to depend on protein synthesis [9]. Thus the early stimulation of RNA polymerase B activity may reflect the stimulation of transcription, resulting in part in the synthesis of a 'lethal protein' as well as a protein inhibitor of glucose transport [10], while the later glucose independent inhibition of this enzyme activity may reflect the disaggregation of chromatin, an early feature of both the steroid- and radiation-induced lysis of lymphoid cells [11].

In contrast, the glucose-dependent inhibition of RNA polymerase A activity by corticosteroids may contribute to their cytostatic action, since the initiation of DNA synthesis in lymphocytes can be prevented by the selective inhibition of ribosomal RNA synthesis [12]. This inhibition of enzyme activity would be expected to have little effect on resting lymphocytes, since the rate of transcription of 45*s* ribosomal precursor RNA is not the rate-limiting step in rRNA synthesis in these cells, but would effectively prevent the increase in rRNA synthesis following growth stimulation, a condition in which the rate of transcription does become rate-limiting [13].

Finally, we consider that the evidence presented here, which indicates that different mechanisms are involved in the inhibition of the activities of the two polymerases by dexamethasone, also raises the possibility that the two effects may occur independently of each other. This possibility is now being investigated for human lymphoid cells, which are known to be resistant to the cytolytic actions of corticosteroids [14], but sensitive to their cytostatic actions [15].

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DISCUSSION

Hamilton. Your work suggests to me that we now have two hormone responsive systems, estrogen acting on RNA polymerase in the uterus and your system, in which it is quite clear that the early stimulation of polymerase B or II may be responsible for later changes in polymerase activities. Could the effects on polymerase which are independent of protein synthesis be mediated by a species of RNA?

Bell. In relation to the inhibition of the B polymerase? Yes, this is a reasonable hypothesis, but the difficulty is in designing experiments to test it since we can not inhibit the stimulation of the B polymerase without inhibiting basal activity as well.