KINETICS OF CORTISOL ACTION ON RNA SYNTHESIS

Chev Kidson[1]

Baker Medical Research Institute,[2]

Melbourne, Australia.

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A variety of experimental evidence suggests that hormones are involved in gene regulation in higher organisms (reviewed by Davidson, 1965). Experiments of two main types have pointed in this direction: the demonstration of enzyme induction by a specific hormone, e.g. cortisone (Feigelson et al., 1962) or estradiol (Ryan et al., 1963) and the demonstration of alterations in RNA synthesis (e.g. Kidson and Kirby, 1964a). The latter experiments have indicated that a number of hormones administered to whole animals give rise to selective changes in synthesis of rapidly-labeled RNA in a tissue such as liver, in relatively short times (5-60 minutes). From these data it has been inferred that hormone action may affect fairly directly DNA transcription into messenger RNA. Other data (Garren et al., 1964) suggest that hormones may regulate the translation step, i.e. synthesis of protein on the RNA template.

The present experiments have been designed to study the time course of action of hormones on the synthesis of RNA as an index of the nature of the reactions involved in hormone

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[1] A.A. Thomas Fellow, Anti-Cancer Council of Victoria.

[2] Postal Address: Commercial Road, Prahran S.1., Victoria, Australia.

regulation of gene expression. An isolated, first-generation cell system (rabbit lymph node cells) has been chosen to permit the use of short time periods and assay of the effects of a single hormone. With this system it has been possible to detect very rapid, cortisol-induced alterations in RNA synthesis.

MATERIALS AND METHODS

Mesenteric lymph nodes from two or more rabbits were pressed through a sieve to remove connective tissue, washed and suspended in 15 vol. Dulbecco medium (Dulbecco and Vogt, 1954) containing 1% glucose. The cell suspension was divided into a number of aliquots to allow internal controls in each experiment. Cortisol, as hydrocortisone sodium succinate, was added in solution where indicated. RNA was pulse-labeled with ^{3}H-uridine (10,000 mc/mM).

To assay RNA synthesis, incubation was terminated by pouring the contents of each flask into an equal vol. of cold Dulbecco medium and 2 vol. of 90% phenol/12% m-cresol/0.1% 8-hydroxyquinoline and shaking immediately. Shaking was continued for 20 min. This released ribosomal (rRNA) and soluble (sRNA) RNA into the aqueous phase (c.f. Kidson and Kirby, 1964b). The insoluble phenolic interphase was washed once with 0.5% naphthalene-1:5-disulphonate and an equal vol. of the phenol mixture, and the aqueous phase added to that from the initial extraction. Ribosomal RNA and sRNA were precipitated from the aqueous phase with ethanol/m-cresol (9:1, v/v), then sRNA was taken up in 3M sodium acetate (2 washes), precipitated with ethanol/m-cresol and both rRNA and sRNA fractions washed with ethanol and dried (c.f. Kirby, 1965). The original phenolic interphase was then extracted with 6% 4-aminosalicylate and an equal vol. of the

phenol mixture, and the DNA, together with residual RNA,was precipitated with ethanol, washed and dried (Kidson, Kirby and Ralph, 1963). With short pulse-times it was found that almost all the newly-synthesized RNA, including messenger RNA, was extracted together with the DNA (Kidson and Kirby, 1964b). Each fraction was assayed by sucrose density gradient sedimentation (Kidson et al., 1963) to assess purity and quality. Radioactivity was counted on a liquid scintillation spectrometer. RNA synthesis is expressed per mg. DNA.

RESULTS AND DISCUSSION

In the lymph node cell system cortisol produces a net decrease in the synthesis of rapidly-labeled RNA and does so optimally at concentrations in the region of 1-5 μg/ml (Fig. 1). In most subsequent experiments cortisol has been used at a concentration of 5 μg/ml. (Fig. 1.)

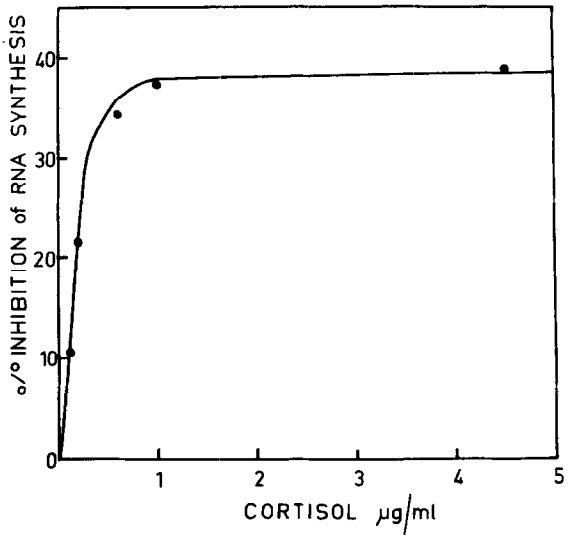

Fig. 1. Effect of cortisol concentration on degree of inhibition of synthesis of rapidly-labeled RNA. 5 min. pulse of cortisol followed by 5 min. pulse of 3H-uridine (10 μc/ml).

The rate of cortisol action on RNA synthesis is shown in Fig. 2. Using a standard ^{3}H-uridine pulse (5 min.), cortisol was added for varying times before the addition of radioactivity. It is evident that even in 1 min. the hormone causes a net decrease in RNA synthesis and that this effect is near-maximal in 5 min. The extent of alteration of RNA synthesis by cortisol varied from one experiment to another, but in lymphoid cells always represented a net decrease, whereas in liver pulsed in situ it always gave a net increase (Kidson and Kirby, 1964a).

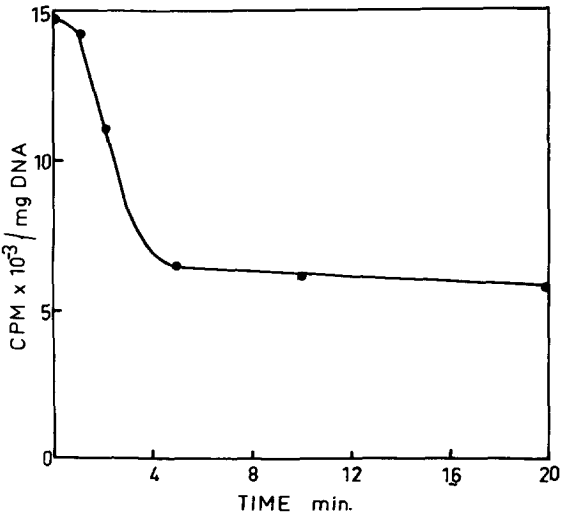

Fig. 2. Time course of inhibition by cortisol of synthesis of rapidly-labeled RNA. Cortisol (5 µg/ml) given for varying times prior to a 5 min. pulse with ^{3}H-uridine (5 µc/ml).

If for a standard (2 min.) pulse of cortisol the pulse-time of ^{3}H-uridine was varied it is evident that even at the shortest times measured (1 min.) there was a net decrease in RNA synthesized (Fig. 3). These experiments confirm those

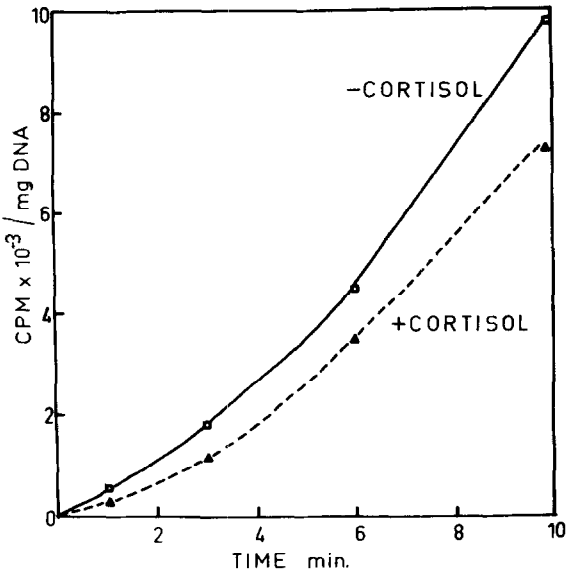

Fig. 3. Effect of cortisol on RNA synthesis over varying periods of time. 2 min. pulse of cortisol (5 µg/ml) prior to ^{3}H-uridine (5 µc/ml) for various times.

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above with varied cortisol pulse-times, showing that the hormone affects rapidly the most newly-synthesized RNA. Much of this RNA isolated together with DNA is probably messenger RNA (Kidson and Kirby, 1964b) but even in very short times some may possibly represent ribosomal RNA precursors (Scherrer et al., 1963). In ^{3}H-uridine pulse-times < 5 min. virtually no radioactivity appeared in the rRNA fraction.

Thus, working with an isolated cell system, it has been possible to confirm the suspicion aroused earlier in whole animal experiments (Kidson and Kirby, 1964a) that at least one hormone, cortisol, acts with extreme rapidity in the regulation of RNA synthesis. This action is readily measurable in 1-2 min. and is presumably initiated in seconds. Delineation of precise kinetics is limited by the experimental techniques used and

currently the very earliest phases of cortisol action are being assessed with the aid of an instrument designed to administer automatically a programmed series of very short pulses.

Although whole animal and isolated cell systems are not directly comparable, it is interesting to note that whereas in rat liver cortisol causes a net increase in the synthesis of rapidly-labeled RNA (Kenney and Kull, 1963; Kidson and Kirby, 1964a), in lymphoid cells there is a net decrease. This correlates with the opposite effects of cortisol on protein synthesis in liver and in thymus noted by Pena et al. (1964) in whole animal experiments and argues against a general action for example, on RNA polymerase, as does the selective nature of cortisol action on rat liver RNA synthesis (Kidson and Kirby, 1964a). A similar selective response is seen in lymphoid cells (Kidson, 1965).

The kinetics of cortisol action on RNA synthesis in the present studies suggest at least superficial analogy with bacterial inducers and co-repressors — in terms of rapidity of response. What is not yet known is whether this rapid action of cortisol is nuclear or cytoplasmic in site.

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REFERENCES

Davidson, E.H., Sci. Am., 212(6), 36 (1965).
Dulbecco, R. and Vogt, M., J. Exp. Med., 99, 167 (1954)
Feigelson, P., Feigelson, M. and Greengard, O., Recent Progr. Hormone Res., 18, 491 (1962).
Garren, L.D., Howell, R.R., Thompkins, G.M. and Crocco, R.M., Proc. Nat. Acad. Sci. U.S., 52, 1124 (1964).
Kenney, F.T., and Kull, F.J., Proc. Nat. Acad. Sci. U.S., 50, 493 (1963).
Kidson, C., in preparation (1965).

Kidson, C. and Kirby, K.S., Nature, 203, 599 (1964a).
Kidson, C. and Kirby, K.S., J. Mol. Biol., 10, 187 (1964b)
Kidson, C., Kirby, K.S. and Ralph, R.K., J. Mol. Biol., 7, 312 (1963).
Kirby, K.S., Biochem. J., 96, 266 (1965).
Pena, A., Dvorkin, B., and White, A., Biochem. Biophys. Res. Comm., 16, 449 (1964).
Ryan, K.J., Meigs, R.A., Petro, Z. and Morrison, G., Science, 142, 243 (1963).
Scherrer, K., Latham, H. and Darnell, J.E., Proc. Nat. Acad. Sci. U.S., 49, 240 (1963).