THE SEQUENTIAL STIMULATION OF URACIL-RICH AND GUANINE-RICH RNA SPECIES DURING CORTISONE INDUCTION OF HEPATIC ENZYMES

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SUMMARY

A mixture of 5-^{3}H-uridine and 8-^{14}C-guanine was employed to evaluate hepatic RNA synthesis at various times following a single administration of cortisone. During the first hour of hormone action, which precedes hepatic enzyme induction, uridine-rich RNA was selectively synthesized; subsequently a hormonal stimulation of guanine-rich RNA synthesis occurred. These findings are compatible with early hormonal stimulation of the synthesis of DNA-like RNA (high A+U) followed by synthesis of ribosomal RNA (high G+C). These double-labeled tracer experiments provide some insight into the biochemical processes underlying hormonal enhancement of hepatic enzyme synthesis and the subsequent augumentation of hepatic ribosomal content.

Although cortisone is known to stimulate RNA synthesis in liver (1-4), the nature of the hormonally enhanced RNA remains controversial and obscure. Some investigators have stressed that cortisone particularly stimulates the synthesis of specific messenger RNA species (5, 6), whereas others have presented evidence of enhanced isotopic incorporation into all subcellular organelles and into all types of RNA: ribosomal, transfer, as well as DNA-like RNA (7-9). These varying inferences arise from differing experimental designs including: (a) the utilization of techniques favoring the isolation of particular types of RNA, (b) measurements made at differing intervals following cortisone administration and often restricted to a single

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time point, and (c) employment of different isotopic precursors in the various experiments. It is generally agreed that m RNA in higher organisms is characterized by high adenylic and uridylic acid content in contrast to r RNA and t RNA which manifest high guanylic and cytidylic acid contents (10, 11). Mindful of these considerations, the present investigation employed the pulse incorporation of a mixture of ^{3}H-uridine and ^{14}C-guanine in an attempt to evaluate both messenger type RNA (m RNA) and ribosomal type RNA (r RNA) synthesis from 10 minutes to 12 hours following glucocorticoid injection.

The time course of hormonal influences on the rates of synthesis of various types of RNA is illustrated in Figure 1-A, in which the effects of cortisone administration on the pulse labeling of total liver RNA with ^{3}H-uridine and ^{14}C-guanine is presented. It is evident that as early as 10 minutes following the administration of 5 mg cortisone acetate per 100 grams body weight into adrenalectomized rats, a more than 50% enhancement in the incorporation of ^{3}H-uridine into hepatic RNA occurred. This stimulated uridine utilization was maintained for 30 minutes, after which it rapidly returned to normal levels. The rate of incorporation of ^{14}C-guanine into RNA was significantly inhibited during the period of enhanced uridine uptake. With the waning of enhanced uridine-rich RNA synthesis, guanine incorporation into RNA augmented, reaching its peak between 3-7 hours, at which time the rate of guanine incorporation into hepatic RNA of cortisonized animals was more than two fold that of control animals Augmented guanine-rich RNA synthesis was maintained for at least twelve hours following cortisone administration. These studies indicate that during the first hour of hormone action, which precedes hepatic enzyme induction, uridine-rich RNA was selectively synthesized; subsequently an

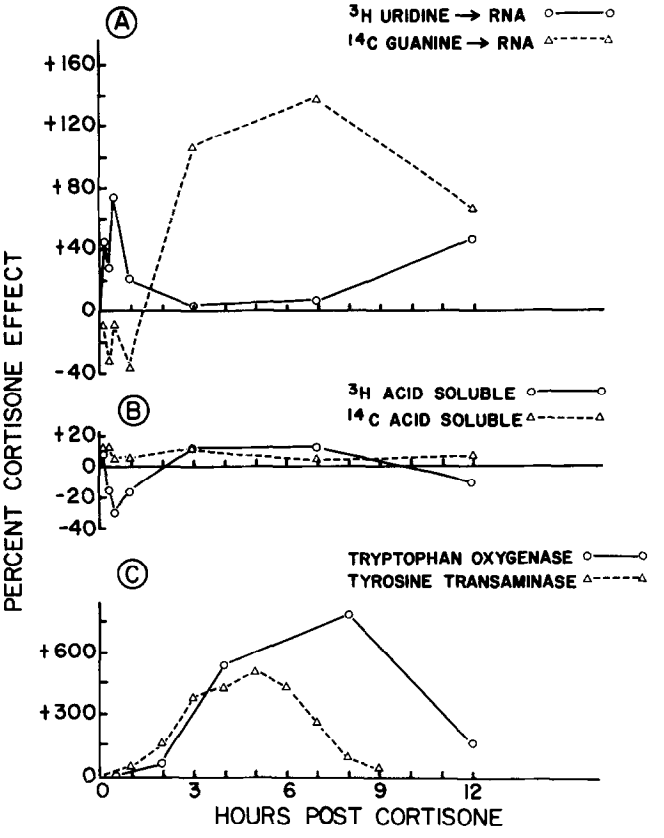

Figure 1. The temporal relationships between cortisone effects on the rates of uridine-rich and guanine-rich RNA synthesis, acid soluble nucleotide metabolism and hepatic enzyme levels. Male Sprague-Dawley rats of 150-200 grams body weight were adrenalectomized and maintained on 1.0% saline, water and Purina Chow ad libitum. Seven days after adrenalectomy, animals were starved overnight and injected interperitoneally with 5 mg per 100 grams body weight of cortisone acetate suspension (Upjohn Co.), the animals were sacrificed at the indicated times thereafter. One ml per 100 g body weight of 0.9% saline solution containing a mixture of 40 μc/ml (5-^{3}H) uridine (Nuclear-Chicago Co., 27.8 c/mM) and 10 μc/ml (8-^{14}C) guanine (International Chemical and Nuclear Co., 12.6 mc/mM) were administered intraperitoneally 10 minutes prior to sacrifice. The livers were immediately chilled to 0° C and rapidly homogenized in 3 volumes of cold 0.27 M $HClO_4$; acid soluble and the RNA fractions were isolated, quantitated; and their radioactivities determined by liquid scintillation procedures with quench correction as previously described (12, 13). The RNA data presented in A are corrected for the acid soluble pool effect shown in B in the manner previously described (13). Each experimental point represents the mean value of 6-10 animals normalized at each time point with a corresponding number of control animals. Tryptophan oxygenase activity was measured on a separate portion of liver by a modification of previously described procedures (12). The tyrosine transaminase data presented was calculated from Garren et al. (22).

hormonal stimulation of guanine-rich RNA synthesis occurred. These findings are compatible with early hormonal stimulation of the synthesis of DNA-like RNA (high A+U) followed by synthesis of ribosomal RNA (high G+C). The present results further suggest that concurrent with the early hormonal stimulation of uridine-rich RNA there may be an inhibited synthesis of guanine-rich r RNA.

It has been previously observed that glucocorticoids markedly elevated the specific activities of acid-soluble nucleotide pools when ^{14}C-glycine or ^{14}C-orotic acid were used as precursors (12). The effect of cortisone administration on the incorporation of ^{14}C-guanine and ^{3}H-uridine into the acid-soluble fraction is depicted in Figure 1-B. During the first hour, there was a 30% decrease in the specific activity of the uridine labeled acid-soluble fraction; beyond this period there was little hormonal effect on the incorporation of either of these two precursors into the acid soluble fraction. In accord with previous findings (13), the presently observed lack of correlation between hormonal effects on acid-soluble precursor metabolism and the enhancement of RNA synthesis indicate these to be independent aspects of hormonal function and exclude the possibility that altered rates of nucleotide synthesis regulate RNA synthesis by a mass action or "pushing" mechanism.

With view towards clarification of the possible causal relationship between m RNA synthesis and hormonal enzyme induction, it is of particular interest to relate the time course of the augmentation in uridine-rich RNA synthesis following cortisone to that of induction of tryptophan oxygenase and tyrosine transaminase. Comparison of Figure 1-C with Figure 1-A reveals that cortisone induction of these two enzymes was preceded by enhanced synthesis of uridine-rich RNA; indeed the

incorporation of uridine into RNA was maximal prior to detectable elevations in the activities of these inducible enzymes. Thus, hormonal enzyme induction is apparently preceded by enhanced transcriptional activity. This conclusion is compatible with other studies in which administration of actinomycin-D prior to hormonal treatment abolished enzyme induction (14) whereas administration of actinomycin-D 1 or 2 hours after the hormone did not interfere with the development of hormonally elevated enzyme levels (15, 16).

Cortisone is known to cause liver hypertrophy; the total liver RNA content increases 28% within 12 hours after a single large injection of cortisone acetate (17), this increase presumably representing largely r RNA. Detection, in the present study, of stimulation of rapidly synthesized uracil-rich RNA prior to stimulation of r RNA synthesis offers the possibility that these processes may be causally related and that the rate of r RNA synthesis may be dependent upon the synthetic rate of a DNA-like (high A+U) RNA species. This view is a variation of a current hypothesis postulating a biosynthetic coupling between transcriptional and translational processes (18-21).

The authors would like to emphasize the general utility of employing, within the same animals, a mixture of ^{3}H-uridine and ^{14}C-guanine, to evaluate the relative rates of synthesis of different RNA species under various physiological or experimental conditions.

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REFERENCES

1. Feigelson, M. and Feigelson, P., in Weber, G. (ed.), Advances in Enzyme Regulation, Vol. III, Pergamon Press, New York, p. 11 (1965).
2. Feigelson, P., Feigelson, M. and Greengard, O., Recent Progress in Hormone Research, 18, 491 (1962).
3. Feigelson, P. and Feigelson, M., in Litwack, G. and Kritchevsky, D. (eds.), Actions of Hormones on Molecular Processes, John Wiley & Sons, Inc., New York, 1964, p. 218.
4. Feigelson, P. and Feigelson, M., in Karlson, P. (ed.), Mechanisms of Hormone Action, NATO Advanced Study Institute, Acad. Press, p. 246 (1965).
5. Kidson, C., and Kirby, K.S., Nature, 203, 559 (1964).
6. Schmid, W., Gallwitz, D. and Sekeris, C.C., Biochim. Biophys. Acta, 134, 80 (1967).
7. Feigelson, M., Gross, P.R. and Feigelson, P., Biochim. Biophys. Acta, 55, 495 (1962).
8. Kenney, F.T., Wicks, W.D. and Greenman, D.L., Cellular & Comp. Phys., 66, Supp. I., 125 (1965).
9. Drews, J. and Brawerman, J., J. Biol. Chem., 242, 801 (1967).
10. Darnell, J.E., Jr., Bacteriological Reviews, 32, 262 (1968).
11. Willems, M., Wagner, E., Laing, R. and Penman, S., J. Mol. Biol., 32, 211 (1968).
12. Feigelson, M. and Feigelson, P., J. Biol. Chem., 241, 5819 (1966).
13. Yu, F.L. and Feigelson, P., Arch. Biochem. Biophys., 129, 152 (1969).
14. Greengard, O. and Acs, G., Biochim. Biophys. Acta, 61, 652 (1962).
15. Mishkin, E.P. and Shore, M.L., Biochim. Biophys. Acta, 138, 169 (1967)
16. Peterkofsky, B. and Tomkins, G.M., Proc. Natl. Acad. Sci., U.S. 60, 222 (1968).
17. Feigelson, P. and Feigelson, M., J. Biol. Chem., 238, 1073 (1963).
18. Stent, G.S., Science, 144, 816 (1964).
19. Byrne, R., Levin, J.G., Bladen, H.A. and Nirenberg, M.W., Proc. Natl. Acad. Sci., 52, 140 (1964).
20. Shin, D.H. and Moldave, K., J. Mol. Biol., 21, 231 (1966).
21. Jones, O.W., Dieckmann, M. and Berg, P., J. Mol. Biol., 31, 177 (1968).
22. Garren, L.D., Howell, R.R. and Tomkins, G.M., J. Mol. Biol., 9, 100 (1964).