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Increased levels of (2′-5′)oligo(A) polymerase activity in human lymphoblastoid cells treated with glucocorticoids

(interferon-induced enzymes/hydrocortisone/cortexolone/growth inhibition)

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ABSTRACT **An enzymatic activity that synthesizes (2′-5′)-oligo(A) from ATP is induced in animal cells treated with interferon. This activity, designated (2′-5′)A polymerase, is also elevated in human lymphoblastoid Daudi and Raji cells treated with hydrocortisone. The polymerase activity increases significantly after 24 hr of treatment and declines when hydrocortisone is removed from the culture medium. The product of the enzyme prepared from hydrocortisone-treated cells is indistinguishable from (2′-5′)oligo(A) synthesized with polymerase of interferon-treated cells either by an endonuclease activation assay or by chromatographic analysis. The increase in (2′-5′)A polymerase is not mediated by secretion of interferon by hydrocortisone-treated cells; less than 1 unit of interferon per ml is present in the culture medium during treatment with this glucocorticoid hormone. Moreover, this increase is related to the concentration of hydrocortisone in the culture medium and is inhibited by the addition of cortexolone. This steroid interferes with the interaction between glucocorticoid hormones and their receptor. Cortexolone has no effect, however, on the induction of (2′-5′)A polymerase by interferon. The synthetic glucocorticoid dexamethasone also increases the polymerase activity. Experiments with inhibitors show that such an increase requires RNA and protein synthesis.**

Two enzymatic activities are induced by interferon in mammalian cells: an oligonucleotide polymerase and a protein kinase (see ref. 1 for additional references.). Both enzymes require double-stranded RNA for activation and can be quantitated in cell extracts by recently developed assays (2, 3). The first enzyme polymerizes ATP into a series of oligonucleotides characterized by (2′-5′)phosphodiester bonds (4) and collectively designated (2′-5′)oligo(A) or (2′-5′)A. These oligonucleotides activate an endoribonuclease that cleaves mRNA (5, 6). The protein kinase phosphorylates the α subunit of initiation factor eIF-2, thus inhibiting initiation of protein synthesis (1). Both these enzymatic activities are elevated in rabbit reticulocytes (7, 8), terminally differentiated cells that synthesize predominantly hemoglobin. The elevated levels of (2′-5′)A polymerase and protein kinase in reticulocytes are not due to an increase of circulating interferon in anemic rabbits (T. Hunter and L. Kronenberg, personal communication). The (2′-5′)A polymerase is also increased in chicken oviduct after withdrawal from stimulation by estrogen (9). In these experiments, the (2′-5′)A polymerase activity remained relatively low for 24 hr after hormone withdrawal and increased gradually with oviduct regression to levels approaching those of reticulocytes or interferon-treated cells (9). These results suggest that the (2′-5′)A polymerase activity may have an important role in cell differentiation (9).

Interferon inhibits the proliferation of several tumor and normal cells in culture (see ref. 10 for additional references). In particular, interferon inhibits DNA synthesis in lymphocytes stimulated by mitogens (11). Kimchi *et al.* (12) reported a similar inhibition of DNA synthesis in lymphocytes stimulated by concanavalin A and treated with relatively high concentrations of (2′-5′)A "core," which does not contain terminal phosphates. These authors suggested that induction of (2′-5′)A polymerase in interferon-treated lymphocytes and production of (2′-5′)A may be responsible for the inhibition of cell growth (12). The proliferation of lymphoid cells is also inhibited by glucocorticoid hormones (see ref. 13 for additional references) and it seemed possible to us that an increase in (2′-5′)A polymerase activity may accompany this growth inhibition. Therefore, we measured (2′-5′)A polymerase after treatment with glucocorticoids in two lymphoblastoid cell lines, Daudi and Raji. These cells are derived from Burkitt lymphomas and do not spontaneously produce interferon (14, 15). The Daudi cells are extremely sensitive to growth inhibition by interferon, whereas the Raji cells are resistant (16). Moreover, the Raji cells are insensitive to the antiviral effect of interferon on vesicular stomatitis virus growth, whereas the Daudi cells are sensitive (16). Because hydrocortisone inhibits proliferation of lymphoblastoid cells (17), we treated these cells with hydrocortisone and observed an increase in (2′-5′)A polymerase activity with time of treatment or concentration of the hormone. This increase cannot be accounted for by the production of interferon, and it is presumably due to *de novo* synthesis of (2′-5′)A polymerase. These results are discussed with regard to the relationship between inhibition of lymphoblastoid cell growth and (2′-5′)A polymerase activity.

MATERIALS AND METHODS

Cell Culture and Treatment. Daudi and Raji cells were cultured in RPMI medium 1640 supplemented with 1 mM glutamine and 20% (vol/vol) calf serum (18). The cells were grown in stationary cultures and treated with glucocorticoids or leukocyte interferon (10^6 units/mg of protein; obtained from K. Cantell, Helsinki) before they reached saturation density at $8–9 \times 10^5$ cells per ml. The cells were diluted with fresh medium to about 4×10^5 cells per ml and treated for the times indicated in figure legends.

DNA Synthesis. Duplicate 0.5-ml aliquots of cell cultures were incubated for 1 hr at 37°C with 5 μCi of [^{3}H]thymidine (1 Ci = 3.7×10^{10} becquerels). At the end of the incubation the cells were centrifuged, resuspended in 0.5 ml of 0.5% NaDodSO$_4$, and treated with 1 ml of 20% (wt/vol) trichloroacetic acid. The precipitate was collected on glass-fiber filters and radioactivity was measured.

Assay of (2′-5′)A Synthesis or Degradation. Cell extracts were prepared by centrifugation of 10-ml aliquots of cultures; the cell pellet obtained was frozen and thawed. The thawed pellet was resuspended in 50 μl of 10 mM KCl, 1.5 mM Mg(OAc)$_2$, and 20 mM Hepes/KOH, pH 7.4. More than 90% of the cells were broken by this treatment, as determined by

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Abbreviation: (2′-5′)A, pppA(2′p5′A)$_n$2′p5′A$_{OH}$ or (2′-5′)oligo(A).

light microscopy. After 10 min, 25 μl of 50% (vol/vol) glycerol in the same buffer was added and the samples were centrifuged for 5 min at 30,000 × g. The assay mixtures for (2′-5′)A synthesis contained 20 μl of the supernatant (about 2.1 mg of protein per ml and 20 A_{260} units), 0.5 μg of poly(I)·poly(C), 125 nmol (0.2 μCi) of [^{3}H]ATP, and the other components previously described (2) in a final volume of 25 μl. The mixtures were incubated for 1 hr at 30°C, and the (2′-5′)A formed was separated by chromatography on DEAE-cellulose as described (2). An aliquot of cell extract was used to determine A_{260}, and the amount of (2′-5′)A synthesized is expressed as nmol of AMP equivalents incorporated into (2′-5′)A per A_{260} unit per hr, as described (9). To synthesize (2′-5′)A of high specific activity for chromatographic analysis on thin-layer plates of polyethyleneimine-cellulose or DEAE-cellulose columns (2), we increased the specific activity of [^{3}H]ATP 60-fold. Conditions for enzymatic digestion of (2′-5′)A have been described (2). The degradation of (2′-5′)A was assayed by incubating cell extracts for 30 min with labeled (2′-5′)A synthesized as described (2). The incubation mixtures were identical to those used for measuring synthesis of (2′-5′)A, except that poly(I)·poly(C) was omitted and 1 μM ^{3}H-labeled (2′-5′)A was substituted for [^{3}H]ATP. Aliquots of the incubations were analyzed by chromatography on DEAE-cellulose to quantitate (2′-5′)A degradation as described (2) and on thin-layer plates to identify the products of degradation as described by Kimchi *et al.* (19).

RESULTS

The initial experiments were carried out with Daudi cells. These cells were treated with 1 μM hydrocortisone, and culture aliquots were taken at the times indicated in Fig. 1 to measure DNA synthesis by 1-hr pulses with [^{3}H]thymidine and (2′-5′)A synthesis by the assay described (2). Synthesis of DNA declined progressively with time of treatment, reaching its lowest value after 48 hr (Fig. 1*A*). Cell division, measured by counting the cells, was also inhibited (data not shown). Synthesis of (2′-5′)A in cell extracts increased with time of treatment, but the greatest increase occurred between 24 and 48 hr of hydrocortisone addition (Fig. 1*A*). This hydrocortisone effect was observed in repeated experiments when the cells were treated immediately after dilution from cultures approaching saturation density. When the cells were washed and incubated in fresh medium without hydrocortisone, synthesis of DNA increased whereas (2′-5′)A synthesis gradually decreased (Fig. 1*B*).

The increased synthesis of (2′-5′)A in extracts of cells treated with glucocorticoids was surprising. Subsequent experiments, therefore, were designed to confirm this finding and to identify this enzymatic activity with that induced in Daudi (unpublished data) and other human cell lines by interferon (2). The synthesis of (2′-5′)A induced by hydrocortisone was absolutely dependent on the presence of activating double-stranded RNA (see legend of Fig. 1) and was optimal at 25 mM $Mg(OAc)_2$, as reported for HeLa cell (2′-5′)A polymerase (2). The products of reactions containing cell extract from hydrocortisone-treated Daudi cells were compared with the products of reactions containing extract from interferon-treated cells by determining their biological activity in an endonuclease activation assay (5) and by chromatography on polyethyleneimine-cellulose plates (data not shown) or on columns of DEAE-cellulose (Fig. 2). The reaction products obtained with these cell extracts and purified by chromatography on DEAE-cellulose (2) activated the endonuclease to an extent comparable to that of (2′-5′)A prepared with extract of interferon-treated HeLa cells (data not shown). The chromatographic analysis of the products digested with phosphatase, phosphodiesterase, and T2 RNase showed identical patterns, indicating that similar compounds were synthesized in the reactions with the different cell extracts. The chromatographic analysis on DEAE-cellulose showed a series of peaks corresponding to the oligomeric series described by Kerr and Brown (4). The predominant species was identified on the basis of its charge with a trimer, but relatively large amounts of tetramer were also synthesized (Fig. 2*A*). When digested with phosphatase, these oligonucleotides lost a −4 charge and were eluted at correspondingly lower salt concentrations (Fig. 2*B*). Similar oligonucleotides were synthesized with extracts of interferon-treated Daudi cells (Fig. 3*C*), suggesting that the same enzymatic activity is elevated in hydrocortisone- and interferon-treated cells. In the following experiments, therefore, we investigated whether the increased formation of (2′-5′)A could be due to synthesis and secretion of interferon after treatment of Daudi cells with hydrocortisone.

Cell cultures treated for 2, 6, or 24 hr with hydrocortisone were centrifuged and the supernatants were assayed for in-

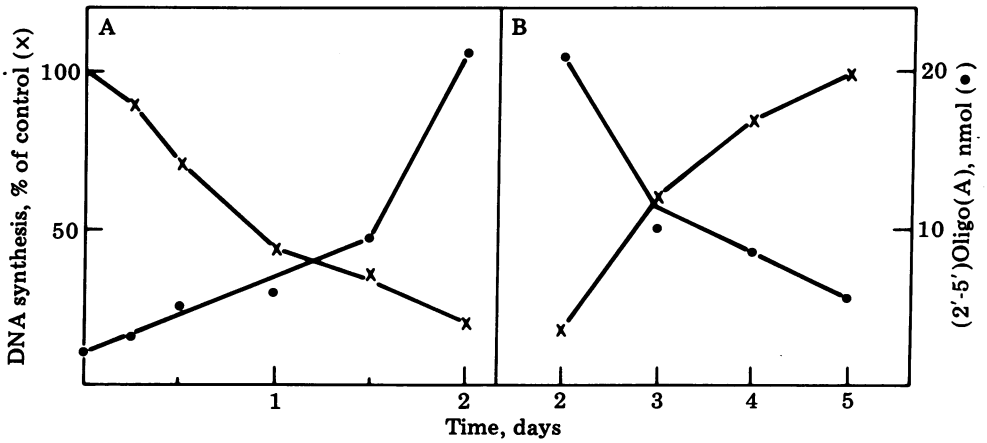

FIG. 1. (*A*) Inhibition of DNA synthesis and increase of (2′-5′)A polymerase activity in Daudi cells treated with 1 μM hydrocortisone. (*B*) Reversal of the inhibition and decline of (2′-5′)A polymerase activity upon removal of hydrocortisone. Cell aliquots were taken at the times indicated to measure DNA and (2′-5′)A synthesis. DNA synthesis is expressed as percent of that of control cultures incubated without hydrocortisone. (2′-5′)A polymerase activity is expressed in nmol of AMP equivalents incorporated into (2′-5′)A in a 1-hr incubation of 25 μl per A_{260} unit of cell extract. Control incubations without poly(I)·poly(C) gave a background of labeled nucleotides eluting with (2′-5′)A corresponding to less than 0.5 nmol of AMP. This background is subtracted from the data shown. (*A*) Hydrocortisone added at time 0; (*B*) cells treated for 48 hr with hydrocortisone were washed twice and incubated in fresh medium lacking hydrocortisone. On subsequent days, additional medium was added to keep the cell density between 4 and 6 × 10^5 cells per ml. The average increase in cell mass was 1.4 per day. Control values for thymidine incorporation were about 50,000 cpm per assay.

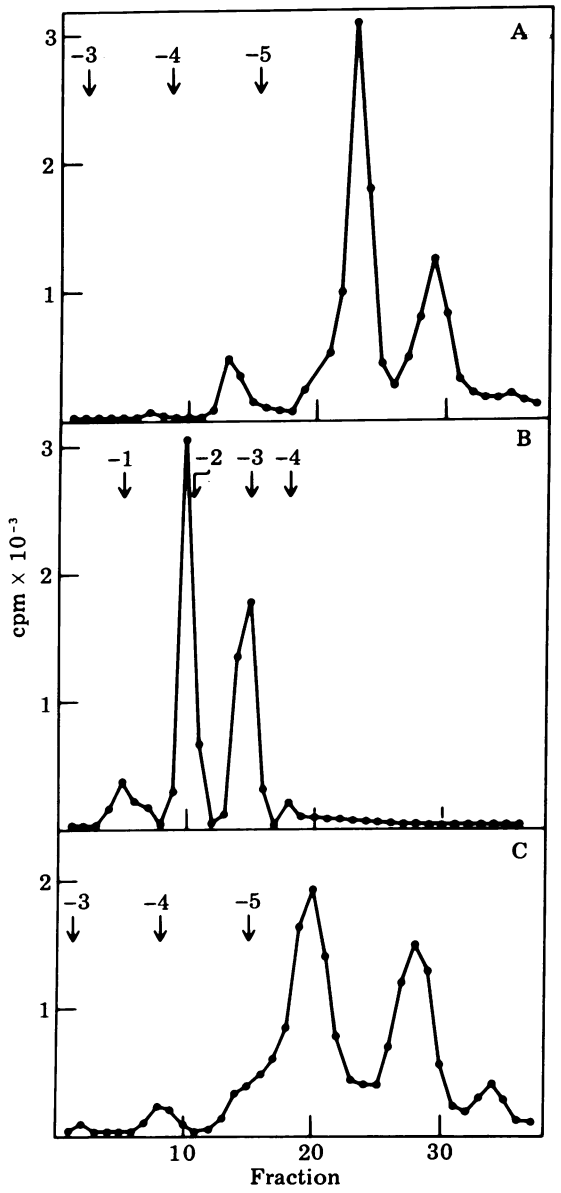

FIG. 2. Chromatographic analysis of (2′-5′)A synthesized by hydrocortisone-treated (*A* and *B*) and interferon-treated (*C*) Daudi cells. The cells were treated for 48 hr with 1 μM hydrocortisone or for 16 hr with 20 units of human leukocyte interferon per ml. Highly labeled (2′-5′)A was synthesized and purified by chromatography on small columns of DEAE-cellulose (2). (*A* and *C*) Aliquots were applied to DEAE-cellulose columns and eluted with 300-ml linear gradients of 50–300 mM NaCl in 7 M urea as described (2); 3-ml fractions were collected and radioactivity was measured. Only fractions 15–52 are shown because no labeled compound was eluted outside these fractions. (*B*) An aliquot of the sample analyzed in *A* was digested with phosphatase, chromatographed, and eluted with a similar 50-ml linear NaCl gradient; 2-ml fractions were collected. The arrows indicate the position of internal markers: −1, A2′p5′A; −2, AMP; −3, ADP; −4, ATP; and −5, p_4A.

terferon content. Monolayers of human foreskin fibroblasts were treated with the supernatant and in parallel with known concentrations of human fibroblast and leukocyte interferon. After 24 hr, the cells were infected with encephalomyocarditis virus; 48 hr later, viable cells were measured by a vital dye uptake method (15). Significant protection of fibroblasts was observed with 1 unit of interferon per ml, whereas no protection was observed with any of the supernatant fractions obtained from hydrocortisone-treated Daudi cells (data not shown). We concluded, therefore, that the increase in (2′-5′)A synthesis is not due to interferon secreted by hydrocortisone-treated cells.

Table 1. DNA synthesis and synthesis and degradation of (2′-5′)A in extracts of Raji cells treated with steroid hormones or leukocyte interferon

Additions	(2′-5′)A Synthesis, nmol AMP equivalents/ A_{260} unit	(2′-5′)A Degradation, pmol AMP equivalents/ A_{260} unit	DNA synthesis, % of control
Exp. 1			
None	0.6	17.0	100
Dexamethasone (1 μM)	15.0	25.2	42
Cortexolone (10 μM)	0.4	19.6	97
Interferon (100 units/ml) + cortexolone (10 μM)	9.8 10.5	20.3 21.8	99 97
Cortisone (1 μM)	1.0	31.2	88
Cortisone (3 μM)	1.1	ND	67
Estradiol (1 μM)	1.2	19.8	72
Estradiol (3 μM)	1.9	ND	48
Exp. 2			
Hydrocortisone (3 μM)	17.5	25.9	ND
+ cycloheximide (50 μg/ml)	1.8	33.0	ND
+ actinomycin D (0.2 μg/ml)	2.0	32.2	ND

In Exp. 1 the cells were treated for 48 hr, whereas in Exp. 2 the cells were treated for 15 hr. Synthesis of DNA was measured as described in the legend of Fig. 1. Synthesis of (2′-5′)A was measured in 25-μl incubation mixtures containing 125 nmol of [^{3}H]ATP; degradation was measured in similar incubation mixtures containing 50 pmol of ^{3}H-labeled (2′-5′)A. ND, not done.

In subsequent experiments we established that synthesis of (2′-5′)A increases in Daudi cell extracts with the concentration of hydrocortisone (Fig. 3*A*). A significant increase was already detectable with the lowest dose tested (30 nM). Synthesis of DNA was also progressively inhibited by increasing concentrations of hydrocortisone, showing a reverse relationship with (2′-5′)A synthetic activity similar to that of Fig. 1.

Further experiments were carried out with Raji cells, which are insensitive to growth inhibition by interferon (16). The synthesis of (2′-5′)A also increased in extracts of these cells after treatment with hydrocortisone or with the synthetic glucocorticoid dexamethasone (Table 1). Extracts of untreated Raji cells synthesized very small amounts of (2′-5′)A (Table 1), whereas extracts of untreated Daudi cells consistently synthesized relatively high levels of (2′-5′)A (Fig. 1). Synthesis of (2′-5′)A also increased in Raji cell extracts after treatment with leukocyte interferon, which did not significantly inhibit DNA synthesis (Table 1).

To determine whether the increased synthesis of (2′-5′)A resulted from the interaction of hydrocortisone with a glucocorticoid receptor, we treated Raji cells with 1 μM hydrocortisone and increasing concentrations of cortexolone, a steroid that blocks the interaction of glucocorticoids with their receptor (20). Cortexolone prevented the increase of (2′-5′)A polymerase and reversed the inhibition of DNA synthesis (Fig. 3*B*). The

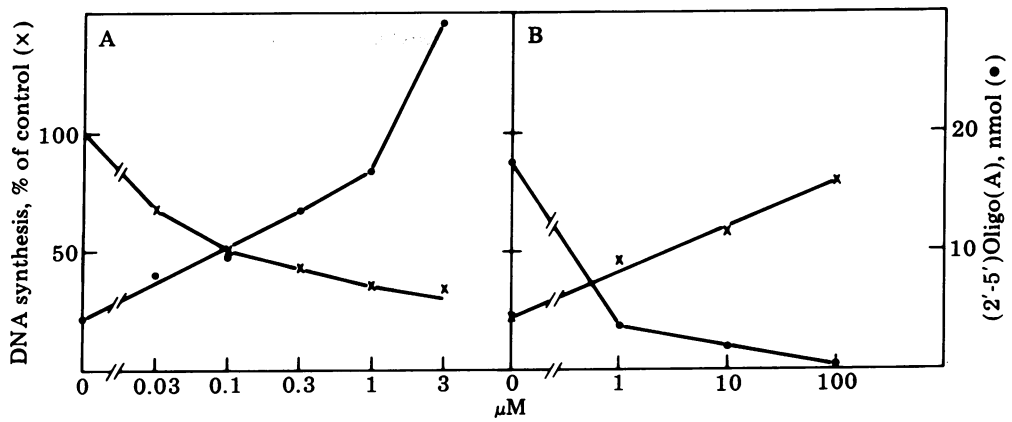

FIG. 3. (*A*) Dose-response of the inhibition of DNA synthesis and increase of (2′-5′)A polymerase to hydrocortisone concentration. Daudi cells were treated for 48 hr with the hydrocortisone concentrations indicated on the abscissa. (*B*) Reversal of the inhibition of DNA synthesis and (2′-5′)A polymerase induction by the antiglucocorticoid cortexolone. Raji cells were treated for 48 hr with 1 μM hydrocortisone and the cortexolone concentrations indicated on the abscissa. DNA synthesis and (2′-5′)A polymerase activity were measured as described in the legend of Fig. 1.

induction of (2′-5′)A polymerase was more sensitive to cortexolone than the inhibition of DNA synthesis, suggesting a differential sensitivity of these processes to this antiglucocorticoid. Moreover, cortexolone had no effect by itself on (2′-5′)A or DNA synthesis nor did it inhibit the increase in (2′-5′)A synthetic activity by interferon (Table 1). Further evidence for a specific effect of glucocorticoids was obtained by treating Raji cells with cortisone or estradiol, which have little or no glucocorticoid activity. Both steroids increased (2′-5′)A synthesis marginally, though they inhibited DNA synthesis (Table 1).

Experiments with inhibitors were carried out to investigate whether RNA and protein synthesis are required for the increase in (2′-5′)A synthetic activity (Table 1). Raji cells were treated with relatively high concentrations of hydrocortisone (3 μM) for a shorter time to limit the toxic effects of the inhibitors. When added with the glucocorticoid hormone, both cycloheximide and actinomycin D largely prevented the increase in (2′-5′)A synthesis.

In the above experiments we detected increased (2′-5′)A synthetic activity in glucocorticoid-treated cells, but our assay could not distinguish between a true induction of (2′-5′)A polymerase and a decrease in enzymatic activities that degrade (2′-5′)A (19, 21, 22). To rule out this latter possibility, we measured degradation of labeled (2′-5′)A under the same conditions of the assay for its synthesis but in the absence of poly(I)·poly(C) and [^{3}H]ATP (see *Materials and Methods*). As shown in Table 1, more (2′-5′)A was degraded in extracts of glucocorticoid-treated Raji cells than in extracts of control cells, indicating that a decreased degradative activity cannot be the explanation for the increased synthesis of (2′-5′)A. Moreover, the amount of (2′-5′)A degraded is relatively small under the assay conditions of (2′-5′)A synthesis. This was shown in experiments in which increasing amounts of (2′-5′)A were added to incubation mixtures identical to those described in Table 1. When the concentration of (2′-5′)A was raised from 1 to 4 μM, the percentage of (2′-5′)A degraded decreased to about one-third the original amount, indicating that in the micromolar concentration range (2′-5′)A is degraded at nearly maximal rate. This relatively low rate of degradation is due to the presence in the reaction mixtures of 5 mM ATP, which was shown to prevent breakdown of (2′-5′)A (2, 22). Because (2′-5′)A is synthesized by extracts of interferon- and glucocorticoid-treated lymphoblastoid cells in nearly millimolar amounts, the degradation of (2′-5′)A may play an insignificant role in the accumulation of these oligonucleotides. This was also established in experiments in which varying percentages of extract of interferon- or glucocorticoid-treated cells were incubated with extracts of untreated Raji cells. Synthesis of (2′-5′)A was proportional to the amount of extract of treated cells over a 4-fold range of concentrations, as previously reported for HeLa cell extracts (2).

Finally, the effect of glucocorticoids on the (2′-5′)A synthetic activity of other cell lines or under different growth conditions was investigated in a preliminary way. No effect was observed in HeLa or L cells treated with either hydrocortisone or dexamethasone. In the lymphoblastoid line Namalva, however, treatment with 1 μM hydrocortisone for 48 hr increased synthesis of (2′-5′)A by cell extracts from a basal level of 1.6 nmol to 6.2 nmol in a standard assay identical to those described for Fig. 1 and Table 1. This increased synthesis of (2′-5′)A, therefore, seems to be characteristic of glucocorticoid-treated lymphoblastoid cells. Even in these cell lines, however, the growth characteristics of the cells during treatment with glucocorticoids play an important role. In experiments with Daudi and Raji cell cultures kept below 5×10^5 cells per ml and at high rate of cell division by addition of fresh medium, the glucocorticoids had little effect on the synthesis of (2′-5′)A by cell extracts.

DISCUSSION

The (2′-5′)A synthetic activity of Daudi and Raji lymphoblastoid cells increases upon treatment with hydrocortisone. The increase is related to the dose of hydrocortisone, time of treatment, and growth characteristics of the cells and is prevented by the antiglucocorticoid cortexolone. This suggests that the interaction of hydrocortisone with a glucocorticoid receptor is required for the increase in (2′-5′)A polymerase activity. Moreover, this effect does not appear to be mediated by the synthesis and secretion of interferon because interferon could not be detected in the medium of Daudi cells treated with hydrocortisone.

The increase in (2′-5′)A polymerase by hydrocortisone indicates that the level of this enzyme can be elevated by hormonal stimuli different from interferon. This is also suggested by the finding that the (2′-5′)A polymerase activity is elevated in reticulocytes and in chicken oviduct after estrogen withdrawal (9). Recently, Blalock and Stanton (23) showed that norepinephrine could induce resistance to viral infection in mouse myocardial cells and proposed that this hormone and interferon may share a common pathway of action in susceptible cells. The antiviral effects of interferon can also be transmitted from treated cells to neighboring cocultivated cells of the same or even different species, possibly by cell-to-cell diffusion of intracellular mediators (24). Different hormones, therefore, may elicit a similar response either by sharing in-

tracellular mediators or by activating convergent pathways of enzyme induction in cells already committed to specific differentiation programs. Reticulocytes and oviduct and lymphoblastoid cells are programmed to respond to different hormonal stimuli, and the increase in (2′-5′)A polymerase activity may be part of the commitment of these cells to specific pathways of differentiation.

The increase in (2′-5′)A polymerase activity by glucocorticoids is possibly related to the growth inhibition of lymphoblastoid cells by these hormones. The increase in (2′-5′)A synthesis was observed in extracts of cells treated toward the end of their growth cycle. Control cultures resume cell division after addition of fresh medium and the (2′-5′)A synthetic activity remains at a basal level, whereas in glucocorticoid-treated cultures the cells do not divide and the (2′-5′)A synthetic activity increases dramatically. This increase requires RNA and protein synthesis, it cannot be accounted for by changes in the level of enzymatic activities degrading (2′-5′)A, and it is of the same order of magnitude as that observed in lymphoblastoid cells treated with interferon (Table 1). These observations suggest that glucocorticoids increase the level of (2′-5′)A polymerase presumably by inducing synthesis of this enzyme, though we cannot exclude the possibility that the glucocorticoids may activate a latent enzyme and that this activation requires RNA and protein synthesis. This effect is specific for glucocorticoids; estradiol or cortisone does not increase (2′-5′)A synthesis by cell extracts (Table 1).

The presence of Epstein–Barr virus genomes in Burkitt lymphoma cells may also play a role in the induction of (2′-5′)A polymerase. An increase in cells containing virus particles is observed in some lymphoblastoid cells treated with hydrocortisone (17). A combination of low temperature and glucocorticoids also enhances the replication of Epstein–Barr virus in producer cell lines (25). Daudi cells are producers whereas Raji cells are nonproducers and are not induced to produce viral particles by hydrocortisone (17). Glucocorticoids also promote transcription of integrated viral genomes in susceptible cells; i.e., dexamethasone increases mouse mammary tumor virus RNA (26) and production of mature virus particles in mammary carcinoma cells (27). This effect is mediated by a specific glucocorticoid receptor (27). It is possible that synthesis of viral RNA is induced by glucocorticoids in lymphoblastoid cells also, but it is not clear how viral RNA could induce (2′-5′)A polymerase without secretion of interferon. Cells that synthesize interferon become antiviral and have presumably elevated levels of (2′-5′)A polymerase only when secreted interferon interacts with the cells (28). Raji cells, however, do not secrete detectable amounts of interferon when treated with hydrocortisone (17) and we did not detect interferon in the culture medium of Daudi cells treated with this hormone.

Glucocorticoid hormones greatly enhance interferon production in the spontaneous interferon producer Namalva lymphoblastoid cells infected with Sendai virus (29). This effect of glucocorticoids may be explained by an increase in (2′-5′)A polymerase activity. A more rapid and enhanced interferon production is obtained by pretreating cells with moderate doses of interferon before virus infection, an effect known as "priming" (see ref. 30 for refs.). Treatment with interferon elevates the (2′-5′)A polymerase activity (1), as does treatment with hydrocortisone in lymphoblastoid cells. It seems possible that an elevated (2′-5′)A polymerase activity may enhance interferon production upon virus infection. The (2′-5′)A polymerase may be part of the recognition mechanism for double-stranded RNA of viral origin; the structural requirements of double-stranded RNA for (2′-5′)A polymerase activation are similar to those for the induction of interferon synthesis (31, 32). An increased level of (2′-5′)A polymerase may be responsible for triggering interferon production earlier and at an enhanced rate.

The relationship between growth inhibition and increase in (2′-5′)A polymerase activity is unclear. Changes in the level of this enzymatic activity with the growth conditions of cells and tissues have been reported by Stark *et al.* (9), but it remains to be established whether such changes are a consequence or a possible cause of growth inhibition. The role of elevated (2′-5′)A polymerase activity in cells undergoing physiological changes in growth characteristics is also obscure. Such changes are of particular interest in view of current studies on the antiproliferative effects of interferon (10).

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