Inhibitory Effect of Cortisol in Vitro on 2-Deoxyglucose Uptake and RNA and Protein Metabolism in Lymphosarcoma P1798

J. M. Rosen, J. J. Fina, R. J. Milholland, and F. Rosen

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203

SUMMARY

Exposure of cells prepared from lymphosarcoma P1798 to 1 X 10^{-6} M cortisol for 3 hr produced a significant inhibition of uridine-3H incorporation into RNA (52%) and of leucine-14C into protein (44%). These effects were minimal in the cortisol-resistant P1798 cells. In the sensitive tumor, cortisol also inhibited the cellular uptake of uridine-3H into the trichloroacetic acid-soluble fraction (33%), whereas the uptake of labeled leucine was only slightly impaired (10%). Although the inhibition of leucine-14C uptake is limited, we cannot exclude this effect as a possible explanation of the inhibition of incorporation of leucine into protein. Studies utilizing a-aminoisobutyric-l-14C acid indicated that the uptake of this nonmetabolizable amino acid was significantly inhibited (20%) only in cells exposed to cortisol 2 hr. When glucose was omitted from the incubation medium, the incorporation of radioactive precursors into RNA and protein was inhibited by 80 to 85%; under the same conditions, inhibition produced by cortisol was only 49 and 63%, respectively, of that observed when glucose was present in the medium. Within 1 hr, the inhibitory action of cortisol on the uptake of 2-deoxyglucose-l-14C was substantially greater than its effect on the incorporation of glycine-14C into nucleic acids and protein. Cycloheximide, at a concentration of 1.4 X 10^{-5} M, inhibited 2-deoxyglucose uptake in a time-dependent fashion somewhat similar to that of cortisol, suggesting that continued protein synthesis may be required for glucose transport into P1798 cells. While the action of cortisol on glucose transport may not be the primary action of the hormone, our data suggest that this effect is important with respect to the hormone-mediated inhibition of nucleic acid and protein biosynthesis in lymphosarcoma P1798.

INTRODUCTION

It is now well established that administration of glucocorticoids to animals or the addition of these steroids to cell suspensions leads to a decreased incorporation of labeled precursors into nucleic acids and protein in a number of lymphoid tissues (3, 10, 17, 23). Many of the recent studies concerned with glucocorticoid action on lymphoid tissue have investigated the in vitro effects of cortisol on rat thymocytes. Using this system, Makman et al. (7) and Nakagawa and White (14) have reported a decrease in nucleic acid and protein precursor uptake and RNA polymerase activity 1 to 3 hr after exposure of thymocytes to cortisol. On the basis of inhibitor studies, Makman et al. (9) reported that continued protein synthesis was required for the inhibitory effect of the steroid on uridine transport into thymocytes but not for the inhibition of RNA synthesis. More recently, they have presented evidence for a cortisol-induced protein inhibitor of several transport and phosphorylation processes in rat thymocytes (8). Munck (13) has demonstrated an early effect of cortisol on glucose uptake or phosphorylation in thymocytes, which was evident 20 to 30 min after the addition of cortisol. Young (27) has concluded that the inhibitory effects of cortisol on the incorporation of labeled precursors into both RNA and protein are dependent on the earlier inhibition of glucose metabolism, possibly mediated by changes in ATP production. Furthermore, the early inhibition of glucose uptake by cortisol was mediated by temperature-sensitive and actinomycin D- and cycloheximide-sensitive steps (12).

Studies in our laboratory have been concerned with the action of glucocorticoids on glucose, nucleic acid, and protein metabolism in lymphosarcoma P1798 (2, 17, 18, 20, 21). The availability of both cortisol-sensitive and -resistant lines of lymphosarcoma P1798 (6) provides an ideal system for correlating the biochemical changes elicited by corticosteroids with tumor regression and dissolution. In a previous publication (17), we reported that glucocorticoids produce an early inhibition of glucose uptake only in cortisol-sensitive P1798 cells and that this effect precedes an inhibition of thymidine incorporation into tumor DNA. It was concluded that a reduced energy supply may be necessary for the cortisol-induced regression of both normal and malignant lymphoid tissues.

The present study was undertaken to determine the temporal relationship between 2-deoxyglucose uptake and RNA and protein metabolism in lymphosarcoma P1798, as well as the effect of glucocorticoids on these processes. A clearly defined time sequence of cortisol action on 2-deoxyglucose uptake and the incorporation of glycine-14C into DNA, RNA, and protein is reported. It is concluded that the cortisol-induced decrease in 2-deoxyglucose uptake occurs...
prior to an inhibition of precursor incorporation into DNA, RNA, and protein. The influence of cycloheximide on 2-deoxyglucose uptake and the inhibitory action of cortisol was also studied. Continued protein synthesis appears to be necessary for normal levels of 2-deoxyglucose uptake into P1798 cells. The studies with cycloheximide suggest that glucocorticoids may be acting to inhibit 2-deoxyglucose uptake by a process related to the action of cycloheximide on protein synthesis. A preliminary report of some aspects of this work has appeared (2).

RESULTS

Effect of Cortisol in Vitro on the Incorporation of Specific Labeled Precursors into RNA and Protein of Lymphosarcoma P1798. As shown in Table 1, exposure of sensitive cells to 1 X 10^(-6) M cortisol for 3 hr produced a marked inhibition of uridine-3H incorporation into RNA (52%) and of leucine incorporation into protein (44%). Minimal changes were seen in the incorporation of leucine into protein in cells obtained from the resistant tumor, although a significant inhibition of uridine incorporation (16%) was evident. Previous studies (16) have also demonstrated a small inhibitory effect of cortisol in vivo on the incorporation of uridine into RNA of the steroid-resistant P1798 tumors. In other experiments, cortisol at concentrations of 10^(-7) to 10^(-5) M exerted a dose-dependent inhibition (35 to 50%) on uridine incorporation into the RNA of steroid responsive cells; this effect was specific for steroids with glucocorticoid activity since, at equivalent concentrations (10^(-6) M), cortisol, aldosterone, 11-deoxycorticosterone acetate, and testosterone inhibited uridine incorporation by 39, 11.3, 12.4, and 5.7%, respectively.

Effect of Cortisol on the Transport of Uridine-3H, Leucine-14C, and AIB-14C into P1798 Cells. An effect of cortisol in rat thymocytes on precursor transport into the TCA-soluble fraction has been reported by Makman et al. (7-10). In previous studies (17, 21), both in vivo and in vitro, we could not detect a significant inhibitory effect of glucocorticoids on thymidine-3H uptake at times when there was a marked reduction of thymidine-3H incorporation into the DNA of cortisol-sensitive P1798 tumors. A comparison of the influence of cortisol on both the uptake and incorporation of uridine-3H and leucine-14C is shown in Table 2. An inhibitory effect of cortisol on uridine-3H uptake, as well as on its incorporation into RNA, was evident (Table 2). However, only a minimal effect on leucine-14C uptake was seen, while leucine incorporation was markedly reduced.

An investigation of the kinetics of uptake of radioactive uridine and leucine has shown that the entry of these precursors into P1798 cells is extremely rapid. Maximal TCA-soluble radioactivity was found within 5 to 10 min after exposure to these labeled precursors. Significant uptake occurred even at 4°. Uptake during a 30-min pulse at 37° may represent both the cellular influx and efflux of radioactivity. The problem of studying transport is further complicated because these precursors are metabolized and incorporated into RNA and protein. So that some of these difficulties could be overcome, an experiment was designed to compare the effect of cortisol on leucine-14C uptake with its effect on the uptake of AIB-14C, a nonmetabolizable amino acid (Chart 1). Exposure to cortisol for 2 hr or longer results in a marked inhibition of AIB-14C uptake into cortisol-sensitive cells. However, under comparable conditions there was minimal effect on leucine uptake, while the incorporation of leucine into protein was significantly decreased.

In studies in Ehrlich tumor cells, Oxender and Christensen (15) have reported that the transport of α-aminoisobutyric acid and leucine is mediated by different systems. Conceivably, in P1798 cells, these 2 amino acids might have independent transport sites, and cortisol affects only 1 of these (α-aminoisobutyric acid) significantly.

Role of Glucose on the Incorporation of Precursors into RNA and Protein. Cortisol-responsive P1798 cells were incubated in the standard glucose or glucose-deficient medium for 3 hr. The omission of glucose from the medium did not result in a significant loss (< 5%) in cell viability, as determined by the trypan blue dye exclusion test (22). The data summarized in Table 3 indicate that omission of glucose material. Uridine-5-3H (5 or 20 Ci/mmmole), L-leucine-U-14C (10 mCi/mmmole) and glycine-U-14C (21.5 mCi/mmmole) were purchased from Amersham/Searle Corp., Arlington Heights, Ill. AIB-U-14C acid (6.4 mCi/mmmole) and 2-deoxy-D-glucose-U-14C (2.29 to 7.85 mCi/mmmole) were obtained from New England Nuclear, Boston, Mass. The complete RPMI 1640 culture medium (11) and its modifications were prepared in this institute. Cortisol was obtained from Mann Research Laboratories, New York, N. Y. A stock solution of cortisol was made in 95% ethanol, and appropriate dilutions of this solution were made with doubly distilled water. Cycloheximide was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, and was dissolved in doubly distilled water.

Methods. Most of the procedures utilized in this paper have been described in a previous publication (17). Fractionation of washed TCA-insoluble material was performed by the method of Wannamacher et al. (26) with the following modifications: RNA was hydrolyzed at 37° for 1 hr in 0.3 N NaOH, DNA was hydrolyzed at 96° for 45 min in 0.5 N HClO4, and the remaining protein fraction was collected on Millipore filters as described previously (17). Results are expressed either as cpm/10^8 cells ± S.E. of the mean or as the percentage of inhibition of the treated mean compared with the control mean. In all experiments, the steroid was present for the total length of time indicated, and pulse labeling was done prior to the end of the total incubation period. Thus, an inhibitory effect of cortisol at 1 hr represents an exposure to the steroid for a total of 1 hr and includes the pulse time. The radioactivity measured in the TCA-soluble fraction is referred to as “uptake,” whereas that found in the TCA-insoluble fraction is designated as that “incorporated” into RNA or protein.

4 In a 14C-labeled compound, U indicates that the compound is uniformly labeled.

5 The abbreviations used are: AIB-U-14C, α-aminoisobutyric-1-14C acid; RPMI, Roswell Park Memorial Institute; TCA trichloroacetic acid.
Effects of cortisol in vitro on the incorporation of precursors into RNA and protein in the cortisol-sensitive and -resistant P1798 cell suspensions

Cell suspensions were exposed to cortisol (1 × 10^{-6} M) for 3 hr. The appropriate labeled precursor was added to 0.5-ml aliquots of both control and cortisol-treated suspensions 30 min prior to the end of the incubation. There were 3 to 5 samples in each group, and the mean values ± S.E. are shown.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Control (cpm/10^6 cells × 10^{-3})</th>
<th>Cortisol (cpm/10^6 cells × 10^{-3})</th>
<th>Change (%)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine-^3H, 1 μCi</td>
<td>17.4 ± 0.6</td>
<td>8.4 ± 0.2</td>
<td>-52 (p &lt; 0.01)</td>
<td>23.0 ± 1.4</td>
</tr>
<tr>
<td>Leucine-^14C, 0.5 μCi</td>
<td>26.1 ± 0.1</td>
<td>14.7 ± 0.5</td>
<td>-44 (p &lt; 0.01)</td>
<td>27.5 ± 0.4</td>
</tr>
</tbody>
</table>

Effect of cortisol on the uptake and incorporation of uridine-^3H and leucine-^14C into cortisol-sensitive P1798 cells

<table>
<thead>
<tr>
<th>Precursor</th>
<th>TCA-insoluble fraction (cpm/10^8 cells)</th>
<th>Change (%)</th>
<th>TCA-soluble fraction (cpm/10^8 cells)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine-^3H, 1 μCi</td>
<td>676 ± 11</td>
<td>-38 (p &lt; 0.01)</td>
<td>3,864 ± 64</td>
<td>-33 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Leucine-^14C, 0.5 μCi</td>
<td>21,088 ± 232</td>
<td>-51 (p &lt; 0.01)</td>
<td>13,574 ± 496</td>
<td>-10 (p &lt; 0.05)</td>
</tr>
</tbody>
</table>

* Conditions were as described for Table 1, except that the 5 Ci/mmole, sample of uridine-^3H, was used.

When glucose was omitted, cortisol affected a slight increase (6.35%) in the uptake of leucine-^14C into the TCA-soluble fraction. However, omission of glucose markedly decreased both uridine-^3H uptake and incorporation into the TCA-soluble and -insoluble fractions in untreated cell suspensions (Chart 2). Thus, glucose is required for the transport and possibly the phosphorylation of uridine in P1798 cells. The inhibitory effect of cortisol on the accumulation of radioactivity from uridine-^3H in the TCA-soluble fraction may be secondary to the effect of the steroid on glucose transport.

Temporal Relationship between the Effect of Cortisol on Deoxyglucose Uptake and the Incorporation of Glycine-^14C into DNA, RNA, and Protein. Previously, we reported that the inhibitory effect of glucocorticoids on glucose uptake appeared to precede its inhibitory effect on the incorporation of thymidine into DNA of lymphosarcoma P1798 (17). However, a clearly defined time sequence of cortisol action on glucose uptake and nucleic acid and protein metabolism has not yet been established in lymphosarcoma P1798. The experiment summarized in Chart 3 compared the effects of cortisol on the uptake of labeled 2-deoxyglucose with its effect on the incorporation of glycine-^14C into DNA, RNA, and protein in steroid-sensitive P1798 cells. 2-Deoxyglucose is a competitive inhibitor of glucose transport, and we have shown that it is not metabolized beyond deoxyglucose 6-phosphate in P1798 cells (17). When compared with untreated controls, a significant inhibition of deoxyglucose uptake of approximately 25% is evident at 1 hr, while changes of less than 10% are seen in the incorporation of glycine into DNA, RNA, and protein. Effects on nucleic acid and protein synthesis, however, are not marked until after 2 hr of exposure to the steroid; in part, this delay might be attributed to a lag.

greatly reduces the incorporation of labeled precursors into RNA and protein (87 and 85%, respectively). Thus, glucose (or another source of energy) appears to be essential for the maintenance of maximal levels of incorporation of precursors into DNA and protein, as well as into DNA (17). Moreover, when glucose is lacking, the inhibitory effect of cortisol on the incorporation of these precursors is also reduced. We had previously shown that omission of glucose minimally affects thymidine-^3H uptake (17) into the TCA-soluble fraction.
Effect of cortisol and glucose on the incorporation of precursors into RNA and protein in the cortisol-sensitive P1798 lymphosarcoma

Table 3

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Medium</th>
<th>Control (cpm/10^6 cells)</th>
<th>Cortisol (cpm/10^6 cells)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine-^3H, 1 µCi</td>
<td>Complete</td>
<td>8,155 ± 447</td>
<td>4,633 ± 160</td>
<td>43 (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>No glucose</td>
<td>1,029 ± 79 (−87%)</td>
<td>814 ± 60</td>
<td>21 (p &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td>Complete</td>
<td>38,874 ± 344</td>
<td>23,960 ± 422</td>
<td>38 (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>No glucose</td>
<td>5,803 ± 123 (−85%)</td>
<td>4,418 ± 99</td>
<td>24 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

Cell suspensions were incubated in RPMI 1640 medium, lacking or containing glucose, and with 1 × 10^-6 M cortisol or vehicle alone for 3 hr. Thirty min prior to the end of the incubation, the labeled precursor was added to 0.5-ml aliquots of each suspension. Each value represents the mean of 3 to 5 individual determinations ± S.E.

Effect of Cycloheximide on 2-Deoxyglucose Transport. It is possible that the altered synthesis of a specific protein mediates the early effect of cortisol on deoxyglucose uptake. Makman et al. (8) have reported that the effects of cortisol on hexose uptake were not evident in the presence of cycloheximide, puromycin, or actinomycin D. Pulse labeling of total cellular protein would not be able to detect cortisol-induced changes in a very small percentage of the total protein. Therefore, studies were undertaken with cycloheximide, an inhibitor of protein synthesis, to determine whether this antibiotic influenced the action of cortisol on glucose uptake P1798 cells. As shown in Table 4, 1.4 × 10^-5 M cycloheximide inhibited the uptake of deoxyglucose-^14C into sensitive P1798 cells by 48% at 3 hr. It also inhibited leucine-^14C incorporation into protein by 91% at this dose as early as 30 min after addition. Cortisol (1 × 10^-6 M) alone or cortisol plus cycloheximide inhibited deoxyglucose uptake by 58% after 3 hr. This did not represent a significant increase over the inhibition (48%) produced by cycloheximide alone.

It is difficult to interpret these data with respect to cortisol action on deoxyglucose uptake, because cycloheximide itself markedly reduced deoxyglucose uptake into P1798 cells. A more complete description of this effect is shown in Chart 4. Cycloheximide at a concentration of 1.4 × 10^-5 M inhibited the uptake of 2-deoxyglucose in a time-dependent fashion quite similar to that of 1 × 10^-6 M cortisol. This inhibitory action did not appear to be due to cell death, since the total cell count and cell viability as shown by the trypan blue dye exclusion test were relatively unchanged at 3 hr.

DISCUSSION

The time course of cortisol action on deoxyglucose uptake and on incorporation of precursors into nucleic acids and protein, and the requirement for glucose for maximal levels of precursor incorporation, suggest that the glucocorticoid-
mediated reduction in glucose transport into P1798 cells may lead to a decrease in the synthesis of these macromolecules. These data support the hypotheses of Munck (13) and Young (27) and provide additional experimental evidence suggesting a temporal relationship between the cortisol effects on glucose uptake and precursor incorporation. Other studies from our laboratory have indicated that cortisol acts on glucose transport across the cell membrane and not on intracellular glucose phosphorylation (19). The effect of glucose appears to be primarily intracellular on thymidine and amino acid incorporation and minimally on the entry of these precursors into the cell. In contrast, uridine uptake does appear to be dependent on a supply of glucose to the cell (Chart 2) and accordingly is also inhibited by cortisol. The transport or phosphorylation of this precursor into P1798 cells, therefore, requires a supply of exogenous substrate to these cells.

Hollander and his colleagues [Ambellan and Hollander (1), Hollander et al. (5), and Stevens et al. (24, 25)] have previously studied the effects of cortisol in vitro on RNA and protein metabolism in lymphosarcoma P1798. They reported changes, such as an increase in RNase activity (1), a decreased incorporation of uridine-3H into both nRNA (25) and rRNA (24), and a reduced ability of microsomes to incorporate labeled amino acids into protein (5) following glucocorticoid administration. The earliest of these effects occurred 6 hr after treatment, and most of these alterations were significant only after 18 hr. However, in a previous publication (17), we reported that cortisol markedly inhibited glucose utilization in the sensitive lymphosarcoma P1798 as early as 4 hr after the animals were treated with the steroid. The time sequence of cortisol effects in vivo is, therefore, similar to those reported herein in vitro.

In studies with rat thymocytes, White and his colleagues [Makman et al. (7, 9, 10)] have previously reported that inhibitors such as cycloheximide and actinomycin D can prevent the inhibitory action of cortisol on glucose uptake, as well as the incorporation of precursors into nucleic acids and protein. An important finding in the present study is that cycloheximide alone, at a dose level comparable to that used in thymocytes, markedly impaired deoxyglucose transport (Chart 4) in P1798 cells. In similar experiments (data not shown), we observed that actinomycin D significantly inhibited the uptake of labeled deoxyglucose into P1798 cells. It is not known whether the effects of these antibiotics on the transport of carbohydrate into P1798 cells is mediated by inhibition of RNA and/or protein synthesis or whether their action on this process involves an effect on the cell membrane independent of inhibition of the synthesis of these macromolecules. Also, it is difficult to determine whether the lack of the inhibitory effect of the hormone in the presence of cycloheximide occurs because protein and RNA syntheses are maximally inhibited, and thus no further effect of the steroid can be measured (Table 4). In this experiment, cortisol plus cycloheximide did not produce an inhibition of deoxyglucose uptake that was significantly greater than that of either treatment alone. Since the concentration of cycloheximide used inhibited protein synthesis by 93%, a further effect of cortisol on protein synthesis might be difficult to demonstrate. Additional studies with lower doses of cycloheximide, as well as with other specific inhibitors of protein synthesis, are necessary to resolve this question. Of interest in this regard are the recent reports by Makman et al. (8) and Hallahan et al. (4) indicating that the action of cortisol on the uptake of hexoses, including deoxyglucose, and other transport processes in rat thymocytes is mediated through the induction of a specific protein inhibitor or inhibitors. On the other hand, our data suggest that inhibition of protein synthesis may be involved in the mechanism by which cortisol influences the transport of deoxyglucose into P1798 cells. Thus, whereas Makman et al. (8) did not observe an inhibitory effect of actinomycin D and cycloheximide on the uptake of deoxyglucose into rat thymocytes, this was clearly the case with P1798 cells (Chart 4). Moreover, these antibiotics, when added simultaneously with cortisol, either prevented or reversed the inhibitory action of the steroid on the transport of several compounds into thymocytes (8) but did not significantly alter the effect of cortisol on 2-deoxyglucose uptake in cells isolated from the P1798 tumor (Table 4). It remains to be determined whether differences in methodology (e.g., in our studies, a complete tissue culture medium was used, whereas the rat thymocytes

### Table 4

<table>
<thead>
<tr>
<th>Drug added</th>
<th>Deoxyglucose uptake (cpm/10⁶ cells)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent only</td>
<td>8289 ± 97</td>
<td>-58</td>
</tr>
<tr>
<td>Cortisol</td>
<td>3442 ± 147</td>
<td>-58</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>4284 ± 863</td>
<td>-58</td>
</tr>
<tr>
<td>Cortisol + cycloheximide</td>
<td>3450 ± 179</td>
<td>-58</td>
</tr>
</tbody>
</table>

*a* Not significantly different from cortisol alone.

*b* Not significantly different from cycloheximide alone.

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Chart 4. In vitro effects of cortisol and cycloheximide on the uptake of 2-deoxyglucose 1-¹⁴C. Cortisol (1 × 10⁻⁴ M), cycloheximide (1.4 × 10⁻⁵ M), or vehicle alone was added to steroid-responsive cell suspensions. Ten min prior to the time indicated, 0.5 μCi of labeled 2-deoxyglucose was added to 0.5-ml aliquots of each sample. Each point represents the mean of 4 samples, and the percentage of inhibition of the treated mean compared with the control mean is shown.
were incubated in a balanced salt solution containing glucose) can account for these striking differences between normal and malignant lymphocytes in their response to certain antibiotics. Several other differences between the effect of cortisol on thymocytes and cortisol-sensitive lymphosarcoma P1798 cells have previously been described (17). To this list may be added the relative lack of effect of cortisol on the uptake of leucine-14C into steroid-responsive P1798 cells, in contrast to the marked decreased transport of this amino acid in treated thymocytes (7, 10). However, despite these differences, the early action of glucocorticoids on glucose transport in the cortisol-sensitive lymphosarcoma P1798 and thymus suggests that this effect may be causally related to hormone-induced regression of both normal and malignant lymphoid tissue.

REFERENCES

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