Specificity and Sensitivity of Cortisol-Induced Changes in Alpha Aminoisobutyric Acid Transport in Human Leukemic Small Lymphocytes and Leukemic Myeloblasts

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ABSTRACT We have examined the in vitro effect of glucocorticoid and nonglucocorticoid steroids on the transport of [3-^{14}C]alpha aminoisobutyric acid (AIB) in lymphocytes from patients with chronic lymphocytic leukemia (CLL), and myeloblasts from patients with acute granulocytic leukemia (AGL). AIB uptake by CLL lymphocytes was markedly inhibited at 1.0 μM (52±2.1%) and slightly inhibited at 0.1 μM (17±3.0%) cortisol. A similar degree of inhibition developed at 50-fold lower concentrations of dexamethasone, indicating that the effect of these steroids on AIB accumulation parallels their glucocorticoid activity in vivo. In contrast, minimal or no inhibition was observed with steroids devoid of glucocorticoid activity (progesterone, testosterone, cortisone). 11-deoxycortisol, a nonglucocorticoid known to impede the binding of cortisol to cellular receptors in animal lymphocytes, failed to inhibit AIB uptake by CLL lymphocytes appreciably, but reduced the effect of cortisol to a statistically significant degree. Hence, cortisol-induced inhibition of AIB transport in CLL lymphocytes is related to its glucocorticoid activity and appears to require initial interaction with glucocorticoid-specific cellular receptors.

In contrast, 1.0 μM cortisol enhanced the accumulation of AIB in AGL myeloblasts from each of five patients studied (mean = 19%, range 7–43%). Neither cortisone nor 11-deoxycortisol stimulated AIB uptake, and cortisol-mediated stimulation was not seen during simultaneous treatment with 11-deoxycortisol, suggesting that this effect of cortisol also represents a specific glucocorticoid effect. The divergent effects of cortisol on amino acid transport in CLL lymphocytes and AGL myeloblasts may explain, in part, the contrasting clinical effects of glucocorticoids administered to patients with these lymphoid and granulocytic hematopoietic malignancies.

INTRODUCTION

Alterations of plasma membrane function may play an important role in mediating the toxic effect of glucocorticoids on lymphoid cells (1–12). Glucocorticoids have been found to impair the transport of various nutrients, including free amino acids, in lymphoid cells from experimental animals. Recently, we have investigated the influence of cortisol on plasma membrane function in human leukemic lymphocytes, in order to determine whether such effects underlie the effectiveness of glucocorticoids in patients with chronic lymphocytic leukemia (CLL)[1] (13). Cortisol in vitro was shown to inhibit the active uptake of alpha aminoisobutyric acid (AIB), a nonutilizable amino acid, in leukemic lymphocytes from 16 patients with chronic lymphocytic leukemia (CLL). Moreover, the extent of inhibition was often highly reproducible in cells from the same patient studied at different times, although it varied widely in cells from different patients.

Munck has pointed out that the metabolic effects of steroids on lymphoid cells may represent (*a*) glucocorticoid actions that can be demonstrated at physiological concentrations and are triggered by the interaction

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[1] *Abbreviations used in this paper:* AGL, acute granulocytic leukemia; AIB, alpha aminoisobutyric acid; CLL, chronic lymphocytic leukemia; ECW, extracellular water; HBSS, Hanks's balanced salt solution; ICW, intracellular water.

of steroids with glucocorticoid-specific cellular receptors, or (*b*) nonspecific effects of steroids that are unrelated to glucocorticoid potency, develop only at high concentrations, and may not be physiologically or pharmacologically significant (14, 15). The characteristics of cortisol-mediated inhibition of AIB transport in leukemic lymphocytes closely resembled the glucocorticoid-specific effects of steroids in animal lymphocytes, in that inhibition developed slowly and required de novo protein synthesis. However, the possibility that cortisol-mediated inhibition of AIB uptake in CLL lymphocytes is nonspecific cannot be excluded, since steroids devoid of glucocorticoid activity were not tested, and high concentrations of cortisol were employed in most instances. The present investigation was undertaken to determine whether cortisol-mediated inhibition of AIB transport in human leukemic lymphocytes represents a glucocorticoid-specific or a nonspecific response.

In contrast with its beneficial influence in patients with CLL (16–18) glucocorticoid therapy is usually ineffective or occasionally deleterious in patients with acute granulocytic leukemia (AGL) (19). To determine whether the divergent clinical responses of leukemic lymphocytes and myeloblasts are mirrored by different metabolic responses to glucocorticoids, we have also studied the effects of cortisol and of nonglucocorticoid steroids on AIB transport in AGL myeloblasts.

METHODS

Cell isolation. Venous blood was obtained from untreated patients with CLL and AGL and anticoagulated with heparin sodium (14 U/ml). White cell and differential counts were performed. 4 vol of blood were then added to 1 vol of 4.0% polyvinylpyrrolidone in Hanks's balanced salt solution (HBSS) and incubated at room temperature for 75–105 min. The leukocyte-rich supernate was removed and centrifuged for 10 min at 200 *g* at room temperature in an International PR-2 centrifuge (International Equipment Company, Needham Heights, Mass.). The cells were resuspended in HBSS, centrifuged again at 200 *g*, and resuspended in HBSS at a final lymphocyte concentration of $1.0–1.2 \times 10^8$ cells/ml and a final myeloblast concentration of $0.3–0.5 \times 10^8$ cells/ml. Red cell contamination was less than 10% as determined by counting 300 cells with a phase-contrast microscope. Over 95% of the leukocytes from patients with CLL were lymphocytes, and over 90% of those from patients with AGL were myeloblasts.

Incubation conditions for AIB uptake. Leukocyte suspensions were added in 0.4 ml portions to 20-ml polyethylene flasks (Rochester Scientific Co., Rochester N. Y.) containing 2 ml of 20% (vol/vol) isologous plasma in HBSS. Isologous plasma was obtained from healthy fasting donors of red cell type AB. Cell suspensions at a final density of 2×10^7 lymphocytes and 7×10^6 myeloblasts/ml were incubated at 37°C in a Dubnoff incubator and shaken at 10 cycle/min in air. Each steroid was added as a solution in absolute ethanol so that the final ethanol concentration was 0.08% (vol/vol). Untreated cell suspensions received an identical amount of ethanol. AIB was added as a 0.1 ml portion to give a final concentration of 10 μM. At the termination of incubation, the contents of each flask were transferred to cooled 2-ml centrifuge tubes (Kimax, Owens-Illinois Inc., Toledo, Ohio) and sedimented at 1000 *g* for 50 s at 2°C. The supernate was immediately decanted, the sides of the tube and the surface of the cell pellet blotted dry, and the cell pellet digested by incubation overnight in a National incubator (National Appliance Co., Portland, Ore.) at 37°C in 0.5 ml of 0.3 M potassium hydroxide (KOH). 0.1 ml of cell digest was added to 10 ml of Bray's solution (20), and its total radioactivity was determined in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The degree of quenching was estimated by external standardization and disintegrations per minute (dpm) were calculated. The protein content of the KOH cell digest was measured by the method of Lowry, Rosebrough, Farr, and Randall (21).

Estimation of intracellular water (ICW). Replicate treated and untreated cell suspensions were incubated for 2 min with [^{14}C]sucrose in place of [3-^{14}C]AIB and sedimented in tared 2-ml centrifuge tubes for 50 s at 1000 *g*. The wet pellet was weighed, dried at 80°C for 18 h, and reweighed. The dry pellet was digested in 0.5 ml of 0.3 M KOH, and the total radioactivity of 0.1 ml of the digest was determined in 10 ml of Bray's solution by liquid scintillography (20). Total pellet water was calculated by subtracting the pellet dry weight from its wet weight. Extracellular trapped water (ECW) was calculated by dividing total pellet radioactivity by the radioactivity per 1.0 ml of the incubation medium. ICW (total pellet water minus ECW) for both lymphocytic and myeloblastic cells was 65–75% of the total cell weight. Steroid treatment for up to 4 h did not alter either ECW or ICW.

Calculation of intracellular amino acid content and expression of transport data. The intracellular accumulation of labelled amino acid per milliliter of ICW (AIB_i) was calculated from the formula:

$$AIB_i = \frac{R_t - (AIB_o \times V_e)}{V_t - V_e},$$

where R_t equals the net radioactivity of the cell pellet, AIB_o the radioactivity per milliliter of incubation medium, V_e the volume of ECW in milliliters, and V_t the total pellet water in milliliters. Results are expressed as V_o, μmol·kg cell water^{-1}·min^{-1}, calculated from the formula:

$$V_o = \frac{AIB_i \times AIB_c}{AIB_o \times \text{time of label in min}},$$

where AIB_c equals the micromolar concentration of [3-^{14}C]-AIB in the incubation medium.

Materials. Cortisol, cortisone,[2] progesterone, and testosterone of highest purity were purchased from Calbiochem, San Diego, Calif., and 11-deoxycortisol[3] from Steraloids Inc., Pawling N. Y. [3-^{14}C]AIB (1 mCi/mmol) and [UL-^{14}C]sucrose (5 mCi/mmol) were obtained from New England Nuclear, Boston, Mass.

RESULTS

Sensitivity of AIB transport in CLL lymphocytes to cortisol in vitro. Exposure to a high concentration of cortisol (10 μM) for 120–240 min has been demonstrated previously to inhibit the active uptake of subsequently

[2] 17α, 21-dihydroxy-4-pregnene-3,11,20-trione.

[3] 17,21-dihydroxy-4-pregnene-3,20-dione.

TABLE I

Initial Rates of AIB Accumulation (V_0) by Cortisol-Treated and Untreated Lymphocytes from Patients with CLL.

Patient	Control V_0*	Cortisol 10 μM V_0	Cortisol 10 μM % inhib.	Cortisol 1.0 μM V_0	Cortisol 1.0 μM % inhib.	Cortisol 0.1 μM V_0	Cortisol 0.1 μM % inhib.
J. A.	9.8	5.3	46	6.3	36	9.0	8
M. S.	6.4	3.1	52	3.4	47	—	—
M. M.	6.0	2.5	58	2.5	58	4.2	30
K. C.	5.3	2.1	60	2.5	53	5.5	0
K. C.	5.1	1.8	65	1.8	65	3.6	29
L. S.	4.7	2.0	57	2.6	45	4.6	2
A. C.	4.3	1.7	60	2.2	49	3.4	21
A. C.	3.2	1.3	59	1.5	53	2.1	34
J. A.	2.8	0.8	71	1.0	64	2.4	14
Mean ±SE	5.3±0.3	2.3±0.2	59±2.4	2.6±0.2	52±3.1	4.4±0.4	17±3.9
		$P < 0.001$		$P < 0.001$		NS	

Lymphocytes from each patient were incubated at 37°C for a total of 260 min without or with cortisol in the concentrations shown. [3-^{14}C]AIB was added to each cell suspension 20 min before termination at a concentration of 10 μM. Each value for V_0 represents the mean of four individual cell suspensions.

* μmol·kg cell water^{-1}·min^{-1}.

added [3-^{14}C]AIB in lymphocytes from patients with CLL (13). In order to determine the sensitivity of CLL lymphocytes to lower concentrations of cortisol, cells from an additional seven patients were incubated with cortisol at low (0.1 μM) and intermediate (1.0 μM) as well as high (10 μM) concentrations for 260 min,

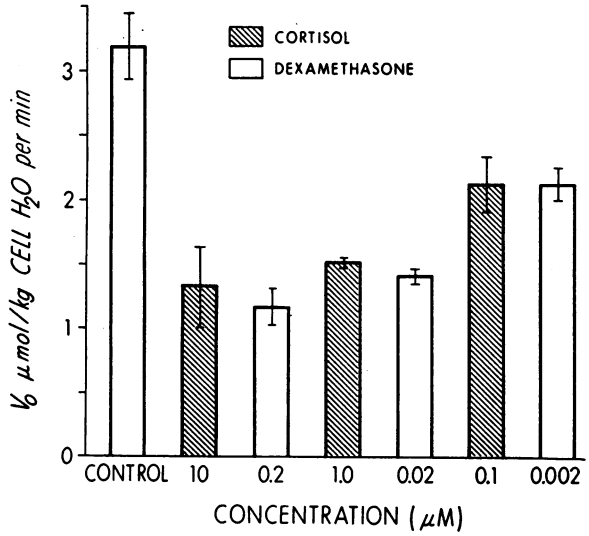

FIGURE 1 Comparison of the V_0 for AIB in CLL lymphocytes from patient A. C. treated with cortisol or dexamethasone. Each value of V_0 for treated cells is significantly ($P < 0.01$) different from control. Incubation conditions are the same as those described in Table I.

and 10 μM [3-^{14}C]AIB was added 20 min before termination. Marked inhibition of AIB accumulation was found in all cell populations treated with high (10 μM) and intermediate (1.0 μM) cortisol concentrations (Table I). A near physiological concentration (0.1 μM) of cortisol was inhibitory in seven of eight studies in six patients. The degree of inhibition was significant ($P < 0.05$) in four of the six patients. Repeated examination of AIB uptake in cells from the same patient performed at intervals of 6 mo revealed no appreciable difference in the response to 10 μM or 1.0 μM cortisol (Table I, patients K. C. and A. C.).

Effects of dexamethasone and steroids devoid of glucocorticoid activity on AIB transport in CLL lymphocytes. We have investigated the possibility that the inhibitory effect of cortisol on AIB uptake is specifically related to its glucocorticoid activity by examining the influence of (*a*) dexamethasone, a synthetic glucocorticoid approximately 25 times as potent as cortisol, and (*b*) a variety of steroids with little or no glucocorticoid activity. Dexamethasone markedly inhibited AIB transport and was as effective at 0.2 μM, 0.02 μM, and 0.002 μM as cortisol at 10 μM, 1 μM, and 0.1 μM, respectively (Fig. 1). Therefore, the relative activities of dexamethasone and cortisol in this system parallel their relative glucocorticoid potencies in vivo (22).

In marked contrast with cortisol, nonglucocorticoids (testosterone, cortisone, 11-deoxycortisol, and progesterone) produced only slight degrees of inhibition even

at a concentration of 10 μM (Fig. 2). Only the effect of 10 μM progesterone was statistically significant when studies in all patients were considered together.

Effect of nonglucocorticoid steroids on cortisol-induced inhibition of AIB transport. Results of recent studies with lymphoid cells from experimental animals indicate that cortisol binds rapidly to specific cellular receptors and that this binding is required for the appearance of subsequent effects on lymphoid cell metabolism (15, 23, 24). The study of cortisol action has been facilitated by the demonstration that cetrain steroids, structurally related to cortisol (e.g., 11-deoxycortisol, progesterone) but with little glucocorticoid activity, compete with cortisol for binding and can block its metabolic effects in vitro (15, 25). Testosterone, which does not bind to these receptors, has not been found to modify the effects of cortisol. In order to determine whether the effect of cortisol on AIB transport in CLL lymphocytes is altered by steroids known to compete for binding to glucocorticoid receptors in animal lymphocytes, we have examined the influence of 11-deoxycortisol, progesterone, and testosterone on the appearance of cortisol-mediated inhibition of AIB uptake. Treatment of CLL lymphocytes with 1.0 μM cortisol resulted in highly significant inhibition (37%) of AIB uptake, whereas a 10-fold higher concentration of 11-deoxycortisol produced only slight inhibition (9%), which was not statistically significant (Table II). Simultaneous treatment of lymphocytes from the same four patients with 1.0 μM cortisol and 10 μM 11-deoxycortisol together resulted in approximately 50% reduction of the cortisol-induced inhibition of AIB uptake (20% vs. 37%) (Table II). While there was no statistically significant difference between the slight inhibition caused by 11-deoxycortisol alone and that caused by cortisol and 11-deoxycortisol together, the degree of inhibition observed with 10 μM 11-deoxycortisol and 1 μM cortisol together was consistently greater and approximates that observed with 0.1 μM cortisol (Table I). In similar experiments, neither progesterone nor testosterone nullified the response of CLL lymphocytes to cortisol (Table III). Although 10 μM progesterone alone was more inhibitory (17%) than other nonglucocorticoids, its modest effect was not additive with the effect of cortisol when both steroids were present together.

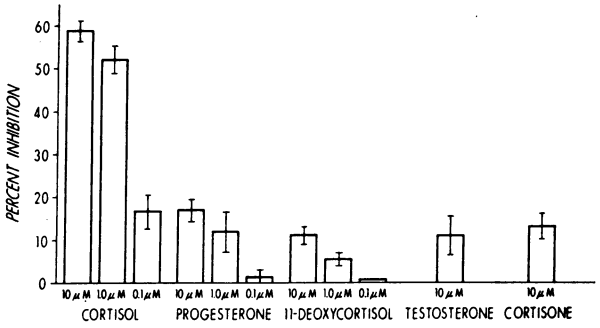

FIGURE 2 Comparison of percent of inhibition of V_o in CLL lymphocytes incubated with various steroids. Incubation conditions are the same as those described in Table I. Each bar represents the mean ±SE of observations in five patients (progesterone), seven patients (11-deoxycortisol and testosterone), and three patients (cortisone).

Effect of cortisol on AIB transport in leukemic myeloblasts. Although the beneficial effect of gluco-

TABLE II

Effect of 11-Deoxycortisol upon Cortisol-Mediated Inhibition of AIB Transport in Leukemic Lymphocytes.

		11-Deoxycortisol 10 μM		Cortisol 1.0 μM		11-Deoxycortisol and cortisol	
Patient	Control V_o*	V_o*	% inhib.	V_o	% inhib.	V_o	% inhib.
H. B.‡	5.7	5.7	0	4.9	14	5.3	7
K. C.‡	4.6	3.6	21	2.8	39	3.7	20
M. S.§	3.7	3.7	0	2.0	46	3.0	19
M. S.§	6.4	5.5	14	3.4	47	4.3	33
Mean ±SE	5.1±0.3	4.6±0.3	8.8±5	3.3±0.3	37±7.7	4.1±0.2	20±5.3

Lymphocytes from each patient were incubated for either 200 or 260 min with the steroids shown. 10 μM [3-^{14}C]AIB was added to each cell suspension 20 min before termination. Each value for V_o represents the mean of four individual determinations. The presence of 11-deoxycortisol significantly (P = 0.05) reduces the cortisol effect in the four patients considered together.

* μmol·kg cell water^{-1}·min^{-1}.

‡ 200 min incubation period.

§ 260 min incubation period.

TABLE III

Effect of Testosterone and Progesterone upon Cortisol-Mediated Inhibition of AIB Transport in Leukemic Lymphocytes.

	Control	Testosterone 10 μM	Cortisol 1.0 μM	Testosterone and cortisol
Mean $V_o \pm$SE*	3.0±0.2	2.9±0.4	1.5±0.2	1.5+0.2
Mean percent inhibition (range)		13 (0–25)	49 (37–64)	47 (42–54)
	Control	Progesterone 10 μM	Cortisol 1.0 μM	Progesterone and cortisol
Mean $V_o \pm$SE*	4.8±0.4	4.1±0.4	3.1±0.3	3.1±0.3
Mean percent inhibition (range)		17 (10–25)	34 (14–47)	35 (25–50)

Lymphocytes from each patient were incubated at 37°C for a total of 200 min with the steroids shown. 10 μM [3-^{14}C]AIB was added to each cell suspension 20 min before termination. Each value for V_o is the mean of observations in three patients.

* V_o = μmoles·kg cell water^{-1}·min^{-1}.

corticoids in patients with CLL (16–18) may be related to their toxic (inhibitory) effects on CLL lymphocyte metabolism, their effect on the course of patients with AGL has been negligible or in some cases deleterious (19). Since inhibition of AIB transport in lymphoid cells is a specific glucocorticoid effect, we have examined this parameter of steroid action in five patients with AGL.

In contrast with studies in lymphocytes, 1.0 μM cortisol was found to stimulate AIB uptake in myeloblasts from each of five patients and in two of these cell populations, the observed stimulation was statistically significant ($P < 0.05$) (Table IV). A similar degree of stimulation was seen in each of four subjects with AGL whose myeloblasts were incubated with 10 μM cortisol (data not shown).

In order to further examine the specificity of the stimulation of AIB transport observed in cortisol-treated myeloblasts, cells from one patient were incubated with 1.0 μM and 10 μM cortisol, 10 μM 11-deoxycortisol, and 10 μM cortisone (Fig. 3). The increase in V_o observed with cortisol alone was not seen with either cortisone or 11-deoxycortisol; rather each hormone decreased V_o slightly. Moreover, the stimulatory effect of cortisol upon AIB uptake was not apparent when cells were incubated with both 1.0 μM cortisol and 10 μM 11-deoxycortisol.

TABLE IV

Initial Rates of AIB Accumulation (V_o) by Cortisol-Treated and Untreated Myeloblasts from Patients with AGL

Patient	Control V_o*	Cortisol 1.0 μM V_o	% stimulation
R. F.	25.5±0.17	29.1±0.85	14 ($P < 0.05$)
L. R.	12.1±0.80	14.4±0.67	19 (NS)
S. B.	11.4±0.24	12.3±0.24	7 (NS)
J. C.	7.70±0.15	8.80±0.40	14 (NS)
M. S.	3.50±0.15	5.00±0.11	43 ($P < 0.01$)

Myeloblasts were incubated at 37°C for 260 min in the presence or absence of 1.0 μM cortisol. [3-^{14}C]AIB was added to each cell suspension 20 min before termination in a concentration of 10 μM. Each value represents the mean ±SE of four separate cell suspensions.

* μmol·kg cell water^{-1}·min^{-1}.

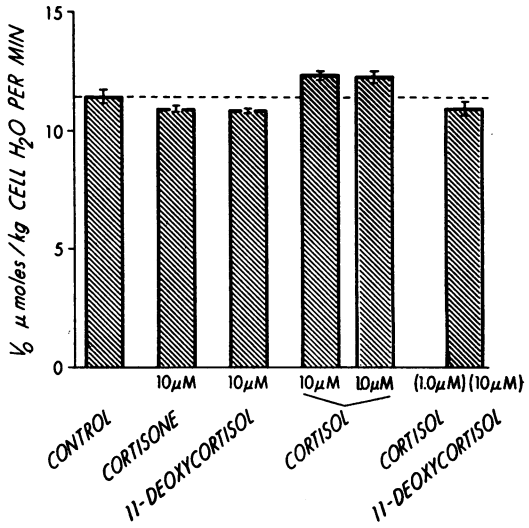

FIGURE 3 Comparison of effects of cortisol and nonglucocorticoid steroids upon AIB transport in AGL cells from one patient, and demonstration of significant ($P < 0.05$) inhibition of cortisol stimulation of AIB transport by 11-deoxycortisol. Each bar represents the mean ±SE of four determinations. Incubation conditions are as described in Table IV.

DISCUSSION

Results of the studies reported herein reveal that a near physiological concentration of cortisol (0.1 μM) in vitro inhibits AIB uptake in lymphocytes from an appreciable proportion of patients with CLL. Although the total concentration of cortisol in normal human plasma ranges from 0.2 to 0.6 μM (26), a significant fraction is bound to serum proteins, and the concentration of free and presumably physiologically active cortisol has been estimated at 20–70 nM (0.02–0.07 μM) (27, 28). Furthermore, exposing untreated cells to very low concentrations of cortisol by the use of plasma (20% vol/vol) in the incubation medium may have provided results which tend to underestimate the sensitivity of CLL cells to cortisol. The consistent inhibition of AIB uptake at 1 μM cortisol may be particularly relevant to the mechanism of the therapeutic effect of glucocorticoids, since equivalent glucocorticoid concentrations are achieved during treatment of leukemic patients.

Three lines of evidence indicate that the inhibitory effect of cortisol on AIB uptake is related to its glucocorticoid activity. First, the activities of dexamethasone and cortisol in vitro parallel their relative glucocorticoid potencies in vivo. Second, steroids devoid of glucocorticoid activity were ineffective or only weakly effective even at very high concentrations. Of interest was the ineffectivenes of cortisone in this system. Cortisone itself is inactive (29–32), and must be converted to cortisol in vivo, primarily in the liver, in order to engender a glucocorticoid response. Our observations suggest that CLL lymphocytes, like animal lymphocytes, do not carry out this conversion. Third, the impairment of cortisol action in the presence of 11-deoxycortisol suggests that binding of cortisol to specific glucocorticoid receptors is required for inhibition of AIB uptake. Studies by Munck and Wira (24), and Rousseau, Baxter, and Tomkins (33) in animal cells have shown that 11-deoxycortisol and progesterone bind to specific glucocorticoid receptors with approximately one third the affinity of cortisol and possess little glucocorticoid activity. Moreover, both steroids competitively inhibit the binding of cortisol in vtiro, and reduce its metabolic effects. The inability of 11-deoxycortisol to completely prevent the appearance of cortisol inhibition would be expected, if 11-deoxycortisol had a lower binding affinity than cortisol for cellular receptors in CLL lymphocytes as in animal lymphocytes. Alternatively, the inhibition produced by cortisol and 11-deoxycortisol together may represent the sum of their nonspecific (nonglucocorticoid) effects. Confirmation of these possibilities will require direct examination of the affinity characteristics of receptor protein from CLL lymphocytes.

In contrast with 11-deoxycortisol, progesterone did not influence the effect of simultaneously added cortisol. Conceivably, progesterone does not compete favorably with cortisol for receptor binding in CLL lymphocytes. The demonstration that progesterone alone inhibited AIB uptake to a greater degree than the other "nonglucocorticoids" (11-deoxycortisol, testosterone, cortisone) is consistent with the observation that progestational agents possess slight glucocorticoid activity in vivo (34, 35). The fact that the effect of progesterone and cortisol together was no greater than that caused by either substance alone suggests a separate site of action for each steroid, or interference with the action of one by the other. We cannot exclude the possibility that progesterone displaces cortisol from binding sites in the plasma added to the incubation medium, since progesterone is known to bind strongly to transcortin (36).

In marked contrast with its inhibitory effect on CLL lymphocytes, cortisol stimulated AIB accumulation in myeloblasts from patients with AGL, and the effect of cortisol on myeloblasts, like its effect on lymphocytes, appears to be related to its glucocorticoid activity. Knospe and Conrad (19) noted beneficial effects from glucocorticoid therapy in less than 10% of adults with AGL, and glucocorticoid administration was frequently associated with increasing leukocytosis and clinical deterioration. Cline and Rosenbaum (37) have found that the ability of cortisol to inhibit [^{3}H]uridine incorporation is greater in lymphoblasts than in myeloblasts. In parallel in vivo studies, administration of glucocorticoids was associated with a striking increase in the number of myeloblasts in one patient, and no effect in nine. Therefore, glucocorticoids may stimulate metabolic events which lead to enhanced production or survival (or both) in some populations of leukemic myeloblasts in vivo.

Whether inhibition of amino acid transport in lymphoid cells and stimulation of amino acid transport in granulocytic cells will prove to be characteristic responses to glucocorticoid hormones in all lymphoid and granulocytic tissues must await studies of normal and other leukemic lymphoid and granulocytic cells.

The study of AIB transport may be useful and convenient in assessing many of the physiological effects of glucocorticoids in intact cells. Although AIB is not utilized, it is transported by a mechanism which subserves the transport of utilizable amino acids (38). Furthermore, studies with animal lymphocytes have revealed parallelisms between glucocorticoid-mediated inhibition of AIB transport and its other effects, such as impairment of sugar, utilizable amino acid and ribonucleoside transport, and of macromolecule synthesis (8, 12, 39). These effects appear to arise indirectly by a glucocorticoid-specific process that depends on de novo protein and RNA synthesis (23), and share similar dose-response relationships. Moreover, alterations in plasma membrane function, as manifested by reduced AIB trans-

port, may be responsible in part for the subsequent lymphocytotoxic effects of glucocorticoids.

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