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Steroid-Stimulated Amino Acid Uptake in Cultured Human Fibroblasts Reflects Glucocorticoid and Anti-inflammatory Potency

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SUMMARY

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In cultured human skin-derived fibroblast monolayers, steroids which exhibit glucocorticoid and anti-inflammatory activity stimulate the uptake of the amino acid analogue α-aminoisobutyric acid (AIB). Stimulation of AIB uptake is maximal after 5 hr of exposure to steroid, persists up to 24 hr in the presence of steroid, and is blocked by cycloheximide. Since the rate of AIB efflux from monolayers is also accelerated in the presence of steroid, the increase in the rate of AIB uptake is attributable to a greater stimulation of the rate of AIB influx. In the presence of steroid the apparent K_m of uptake of AIB is reduced without an appreciable effect on the apparent V_{max} of uptake. Steroid analogues lacking an 11β substituent (e.g., prednisone, cortisone) neither stimulate the uptake of AIB nor block the action of active analogues. The stimulation by active derivatives is concentration-dependent, and compounds can be ranked in potency according to their ED_{50} values for stimulation of AIB uptake: cortisol (44 nM) < prednisolone (22 nM) < triamcinolone (13 nM) < beclomethasone (7 nM) = betamethasone (7 nM) < dichlorisone (4 nM) < dexamethasone (2 nM). The order of potencies broadly parallels the relative daily oral replacement doses of these compounds in humans and indicates a high intrinsic potency of the 21-alcohol chlorinated glucocorticoids, dichlorisone and beclomethasone.

INTRODUCTION

The diverse effects *in vivo* of glucocorticoids, which stimulate processes such as protein and nucleic acid synthesis in tissues like liver, while inhibiting the same events in other tissues, have long been recognized (1). Cells which respond *in vitro* to physiological concentrations of glucocoroticoids also exhibit these contrasting differences in glucocorticoid action (2-6). For example, the growth of mouse L929 cells is inhibited by 11β-hydroxycorticosteroids (7), whereas DNA synthesis is stimulated by glucocorticoids in cultured mouse 3T3 fibroblasts (8, 9). Studies with human cells have revealed that glucocorticoids inhibit DNA synthesis in lectin-stimulated peripheral white blood cells (10) and that skin-derived fibroblasts possess

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cytoplasmic glucocorticoid receptors (11).

The cultured fibroblast provides an attractive tissue sample for studies of the genetics and mechanisms of steroid action in man. Samples are readily available, can be propagated for repeated measurements of hormone action, and can be studied in an environment removed (e.g., by several generations of culture) from that of the donor. Whereas studies of steroid binding to cytoplasmic receptors appear to be feasible for human-derived fibroblast samples (11), fibroblast responsiveness has not, to date, been used to assess steroid action in human-derived samples. It is a considerable advantage in interpreting experimental data to be able to measure biological responsiveness in parallel with studies of receptor binding, as has been achieved for measurements with polypeptide hormones (12–15). The success in evaluating receptor function for polypeptide hormones in cultured human fibroblasts (16–22) prompted the present study evaluating the action of glucocorticoids under similar conditions of culture.

In this communication it is demonstrated that synthetic and naturally occurring glucocorticoids at low concentrations (1 μM–1 nM) stimulate the rate of uptake of α-aminoisobutyrate in cultured human fibroblast monolayers with relative potencies which parallel previously measured estimates of the glucocorticoid and anti-inflammatory activity of the steroids; a strict requirement for an 11β substituent (hydroxy or chloro) on the steroid ring is observed. The studies provide a basis for the further analysis *in vitro* of glucocorticoid action in humans.

MATERIALS AND METHODS

Chemicals. Cortisol and cortisone (all steroids used were obtained as the 21-alcohol), crystalline bovine albumin (Pentex), α-aminoisobutyric acid, puromycin, and cycloheximide were obtained from Calbiochem; dexamethasone, from Merck; and aldosterone, betamethasone, cholesterol, deoxycorticosterone, dihydrotestosterone, testosterone, and triamcinolone, from Sigma. Prednisolone was a kind gift from Dr. C. H. Robinson, Department of Pharmacology and Experimental Therapeutics, Johns Hopkins University School of Medicine; beclomethasone, dichlorisone, 16β-methylprednisone, and prednisone were generously provided by Dr. T. L. Popper, Schering Corporation. Standard solutions of all steroids were prepared (0.3–3 mg/ml) in 95% ethanol and stored at −20°. Epidermal growth factor was prepared from fresh frozen mouse submaxillary glands by the method of Savage and Cohen (23). ^{3}H-Labeled α-aminoisobutyrate (500 mCi/mmole) was obtained from ICN, and ^{3}H-labeled thymidine (6.7 Ci/mmole), from New England Nuclear. Chemicals used in the preparation of tissue culture media, unless otherwise indicated, were products of North American Biologicals, Miami, Fl.

Cell culture. Fibroblast samples (mycoplasma-free), derived from adult male donor skin biopsies, were provided by Dr. E. L. Schneider, National Institutes of Health Gerontology Research Center, Baltimore, and were routinely propagated in a humidified atmosphere of 5% CO_2 in air at 37° as monolayer cultures in 75-cm^2 plastic T-flasks in antibiotic-free Eagle's (24) minimal essential medium with Earle's (25) balanced salts, supplemented with 200 mM glutamine and 10% (v/v) fetal bovine serum (Gibco; by immunoassay it had a cortisol content of 50 ng/ml). Cells were subcultured by trypsin (Microbiological Associates) dispersion, both into T-flasks and into 24-well, 1.5-cm-diameter multidish trays (Linbro, New Haven) as previously described (18, 21, 22). Monolayers (1.5-cm diameter in multidish trays) used for the assay of steroid action were plated with culture medium (1 ml) containing 10% (v/v) serum, and were refed twice at 2-day intervals with medium containing 5% (v/v) serum; confluent cell monolayers were responsive to steroids from 4 to at least 14 days after reaching confluency in the final change of growth medium.

Uptake of AIB[2] *and thymidine incorporation*. Measurements of AIB uptake were

[2] The abbreviations used are: AIB, α-aminoisobutyric acid; TEA, Earle's buffer containing 25 mM Tris-HCl, pH 7.4, and 0.1% (w/v) crystalline bovine albumin.

performed on 1.5-cm fibroblast monolayers (50–100 μg of cell protein) essentially as previously described (18), with several modifications as outlined below and in the table and figure legends. Appropriate dilutions of steroids [stock solutions in sterile Earle's buffer containing 25 mM Tris-HCl, pH 7.4, and 0.1% (w/v) cyrstalline bovine albumin] were added to the 1-ml growth medium in which cells had reached confluency ("conditioned medium"); the final concentration of ethanol, which by itself was without effect, was always less than 0.1% and usually well below 0.02% (v/v). Cells were then incubated with steroid for varying periods of time at 37° in a 5% CO_2 humidified atmosphere, after which the monolayers were rinsed free from growth medium by two changes (1 ml for 0.5 hr, then 0.5 ml for 2 hr at 37°, 5% CO_2 humidified atmosphere) of Earle's buffer containing 25 mM Tris-HCl (pH 7.4), 0.1% (w/v) crystalline albumin, and an appropriate concentration of steroid. After the final change of buffer (2 hr of incubation) the multidish tray was moved to a water bath at 37° under an atmosphere of room air, and the uptake of [^{3}H]AIB (final concentration, 7.4 μM) was measured during a 12-min period. Cells were rinsed with three 2-ml portions of ice-cold buffer, solubilized for 10 min at 70° in 0.4 ml of 0.2 N NaOH, and neutralized with 0.4 ml of 0.2 N HCl, and radioactivity was measured by scintillation counting (25% efficiency; average, 400 cpm/pmole of AIB) in 10 ml of Hydromix (Yorktown). The incorporation of [^{3}H]thymidine (1 μCi/ml) into acid-insoluble material was measured as previously described (18, 24) after a 2-hr pulse begun 22 hr after the addition of steroid or epidermal growth factor.

RESULTS

Stimulation of AIB uptake by steroids. In the presence of steroids with recognized glucocorticoid and anti-inflammatory activity the rate of uptake of AIB was increased (Fig. 1 and Table 1). As has been previously observed (17), and as indicated by the data in Table 1, the baseline uptake of AIB varied from one preparation of cells to another, as did the degree of stimulation of AIB uptake (1.2–2.2-fold). However, irrespective of the baseline level of uptake of AIB, stimulation of uptake by glucocorticoids was reproducibly observed in responsive cell lines. Analogues lacking an 11β substituent (hydroxyl or chloro) or otherwise lacking established glucocorticoid activity did not stimulate AIB uptake. In experiments similar to those in Table 1, compounds active in stimulating AIB were aldosterone, beclomethasone, betamethasone, cortisol, dexamethasone, prednisolone, and triamcinolone; inactive com-

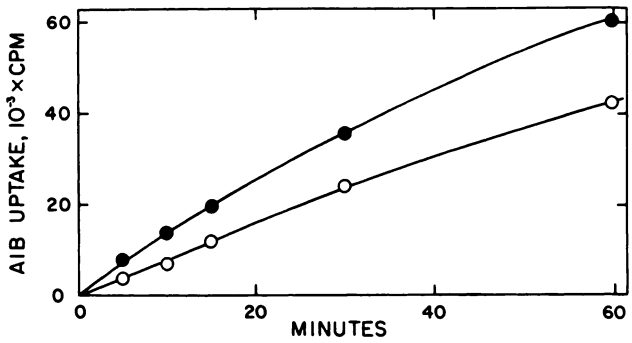


FIG. 1. *Stimulation of AIB uptake by prednisolone*

Monolayers in conditioned medium were incubated at 37° in a humidified, 5% CO_2 atmosphere in the presence (●——●) and absence (○——○) of prednisolone (78 ng/ml) for 17 hr, at which time the medium was changed to amino-acid free buffer (TEA, 1 ml) with or without prednisolone, and incubation was continued for 0.5 hr. The medium was changed a second time (0.5 ml of TEA with or without prednisolone), and monolayers were incubated for 2 hr before the multidish tray was moved to a water bath at 37° under room air. [^{3}H]AIB (7.4 μM, final concentration; 400 cpm/pmole) was immediately added, and the uptake per monolayer (average of duplicate measurements) was determined at timed intervals as described in MATERIALS AND METHODS.

TABLE 1

Stimulation of AIB uptake by steroids

Confluent monolayers in conditioned medium were exposed to reagents at 37° in a 5% CO_2 atmosphere either for 20 hr (experiments 1 and 2) or for 3.5 hr (experiment 3), after which the medium was changed to TEA buffer (1 ml with or without reagents) and incubation was continued for 0.5 hr. The medium was changed a second time (0.5 ml of TEA buffer with or without reagents), and monolayers were incubated for 1.5 hr more before measurement of the uptake of AIB as described in MATERIALS AND METHODS. Values represent the mean uptake of AIB per monolayer ± standard deviation for three replicate measurements; the specific activity of AIB was 400 cpm/pmole.

Additions	AIB uptake
	cpm
Experiment 1	
None	12,210 ± 390
Prednisone (78 ng/ml)	13,270 ± 600
Prednisolone (78 ng/ml)	22,300 ± 1,460
Cortisone (118 ng/ml)	12,900 ± 500
Cortisol (118 ng/ml)	21,970 ± 1,350
Testosterone (78 ng/ml)	12,660 ± 1,190
Dihydrotestosterone (78 ng/ml)	13,154 ± 630
Experiment 2	
None	15,570 ± 550
Prednisolone (39 ng/ml)	26,060 ± 600
Prednisolone (39 ng/ml) + prednisone (784 ng/ml)	25,030 ± 170
Experiment 3	
None	4,770 ± 880
Prednisolone (154 ng/ml)	7,760 ± 740
Triamcinolone (129 ng/ml)	7,480 ± 850
Triamcinolone (129 ng/ml) + prednisolone (154 ng/ml)	7,640 ± 650

pounds (at concentrations of 78 ng/ml or greater) were cholesterol, cortisone, deoxycorticosterone, 16β-methylprednisone, prednisone, testosterone, and dihydrostestosterone. Furthermore, an inactive compound such as prednisone did not inhibit the cellular response to the corresponding 11β-hydroxy analogue, prednisolone (experiment 2, Table 1).

The maximal response in a given cell preparation did not differ significantly for the different active compounds; in addition, maximally effective concentrations of one steroid did not further stimulate cells already maximally stimulated by a second steroid (experiment 3, Table 1). In contrast, insulin and epidermal growth factor caused a further increase in the rate of AIB uptake in cells exposed to maximally effective concentrations of prednisolone (Table 2); again prednisone was without effect.

Not only was the net rate of AIB uptake increased in the presence of glucocorticoid, but the efflux of AIB from cells was slightly faster in the presence than in the absence of steroid (Fig. 2). It may be concluded that the glucocorticoid must increase both the influx and efflux rate of AIB, with a greater effect on influx, so that the net effect is increased cellular uptake.

Lack of response of some cell lines. It was observed that some cell lines failed to

TABLE 2

Combined effects of prednisolone, epidermal growth factor, and insulin on AIB uptake

Confluent monolayers in conditioned medium were exposed at 37° (humidified 5% CO_2 atmosphere) to steroids for 17 hr, at which time the medium was changed to an amino acid-free buffer (1 ml of TEA), with or without steroids, and the incubation was continued for 2 hr. The buffer was changed a second time to 0.5 ml of TEA containing the appropriate amounts of all reagents (indicated below), and cells were incubated for 2.5 hr, at which time the uptake of AIB was determined as described in MATERIALS AND METHODS. In these experiments the specific activity of AIB was 400 cpm/pmole. Values represent the mean AIB uptake per monolayer ± standard deviation for three replicate determinations.

Additions	AIB uptake
	cpm
Experiment 1	
None	15,700 ± 2,750
Prednisone (1961 ng/ml)	15,320 ± 1,370
Prednisolone (1961 ng/ml)	24,110 ± 1,860
EG factor[a] (6.9 ng/ml)	21,380 ± 1,820
EG factor (6.9 ng/ml) + prednisone (1961 ng/ml)	20,950 ± 2,320
EG factor (6.9 ng/ml) + prednisolone (1961 ng/ml)	35,200 ± 1,730
Experiment 2	
None	18,130 ± 1,340
Prednisone (1961 ng/ml)	17,070 ± 1,450
Prednisolone (1961 ng/ml)	28,750 ± 1,400
Insulin (39 ng/ml)	24,880 ± 1,250
Insulin (39 ng/ml) + prednisone (1961 ng/ml)	28,980 ± 1,400
Insulin (39 ng/ml) + prednisolone (1961 ng/ml)	37,570 ± 1,710

[a] Epidermal growth factor.

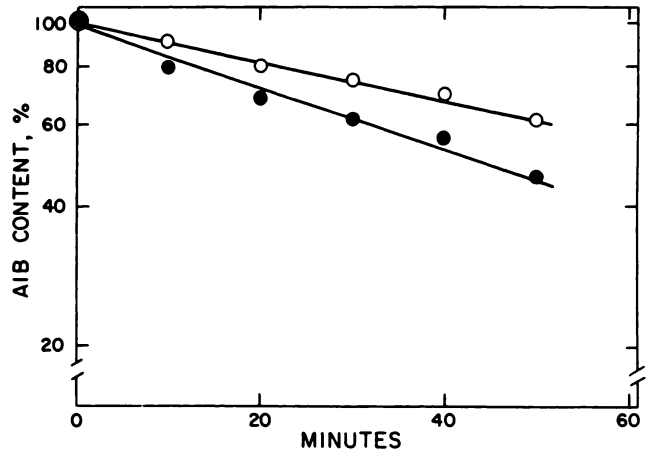


FIG. 2. *Efflux of AIB from fibroblast monolayers*

Monolayers with or without added prednisolone (78 ng/ml) were treated as described in the legend to Fig. 1. AIB (7.4 μM, final concentration; 400 cpm/pmole) was then added, and uptake was allowed to proceed for 1.5 hr at 37°. The supernatant was then replaced with buffer free from AIB, with or without prednisolone, and the monolayers were rinsed at timed intervals. The amount of AIB remaining was determined after solubilization, and the AIB content was expressed as a percentage of the amount present when efflux originated. At the beginning of efflux there were 114 pmoles of AIB per monolayer (45,500 cpm) in untreated monolayers (○——○) and 187 pmoles of AIB per monolayer (74,900 cpm) in monolayers treated with prednisolone (●——●).

respond (with increased AIB uptake) to glucocorticoids. Of eight independently obtained skin samples examined over a 10-month period, two fibroblast cell lines consistently failed to exhibit an increased rate of AIB uptake in response to steroid. Cell lines which, after the primary explant from skin, responded to glucocorticoids in this manner continued to do so, both before and after preservation at liquid nitrogen temperatures. The cell line consistently exhibiting the largest increase in AIB uptake in response to prednisolone was used for the majority of experiments; other steroid-sensitive cell lines yielded similar results in confirmatory experiments.

Rate of onset of steroid action. The stimulation of AIB uptake was not apparent immediately after the addition of glucocorticoid (Fig. 3). Maximal stimulation was observed after about 5 hr of exposure to steroid; stimulation persisted for up to 24 hr in the presence of steroid. No appreciable differences (ED_{50} values) were observed in dose-response curves for the glucocorticoids after 4 hr or 17 hr of exposure to steroid; for convenience, most dose-response curves were obtained from measurements of AIB uptake performed 17 hr after the addition of glucocorticoid, at which time the steroid effect had apparently stabilized (Fig. 3). The addition of either cycloheximide or ouabain along with glucocorticoid abolished the glucocorticoid-mediated increase in AIB uptake measured after 4 hr of exposure to steroid; puromycin only partially inhibited the glucocorticoid-mediated increase in AIB uptake (Table 3).

Dose-response relationships for glucocorticoids. While the degree of stimulation of AIB uptake by glucocorticoids varied somewhat (1.2–2.2-fold), as mentioned above, dose-response curves of the kind depicted in Fig. 4 were readily obtained, with reproducible ED_{50} values which were independent of the degree of increase in AIB uptake (Table 4). In order of increasing molar potency, the active analogues can be ranked: cortisol < prednisolone < triamicinolone < beclomethasone = betamethasone < dichlorisone < dexamethasone. The order of potency indicated by the ED_{50} values for stimulation of AIB uptake parallels previous estimates *in vivo* of the relative glucocorticoid and anti-inflammatory activities of the steroid analogues (26).

Effect of steroids on AIB kinetics. The

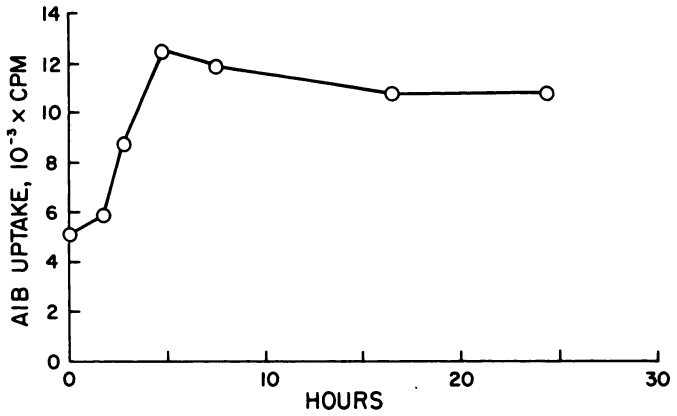


FIG. 3. *Time course of onset of prednisolone-stimulated AIB uptake*

Monolayers in conditioned medium were exposed to prednisolone (78 ng/ml) for varying periods at 37° in a 5% CO_2 humidified atmosphere, after which the medium was changed to amino acid-free buffer (1 ml of TEA with prednisolone, except for zero-time control) and the incubation was continued for 0.5 hr. The medium was changed a second time (0.5 ml of TEA with prednisolone, except for zero-time control), and the incubation was allowed to proceed for 1.5 hr more before the measurement of uptake of AIB (final concentration, 7.4 μM; 400 cpm/pmol) during 12-min period. The abscissa shows the total time of exposure to prednisolone.

TABLE 3

Compounds that modify steroid-stimulated AIB uptake

Confluent cell monolayers in conditioned medium were incubated at 37° in a 5% CO_2 humidified atmosphere with reagents as indicated for 3 hr, at which time the medium was changed to an amino acid-free buffer (1 ml of TEA, with appropriate reagents) and incubation was continued for 0.5 hr. After a second change of buffer (0.5 ml of TEA with or without reagents) cells were incubated for 1.5 hr, at which time the uptake of AIB during a 12-min pulse was measured as described in MATERIALS AND METHODS. Values represent the mean uptake per monolayer ± standard deviation for three replicate measurements; the specific activity of AIB was 400 cpm/pmole.

Additions	AIB uptake
	cpm
Experiment 1	
None	3010 ± 270
Prednisolone (78 ng/ml)	5700 ± 50
Cycloheximide (24,510 ng/ml)	2220 ± 90
Prednisolone (78 ng/ml) + cycloheximide (24,510 ng/ml)	2030 ± 270
Puromycin (19,610 ng/ml)	3000 ± 120
Prednisolone (78 ng/ml) + puromycin (19,610 ng/ml)	4960 ± 250
Experiment 2	
None	5258 ± 340
Dexamethasone (308 ng/ml)	6425 ± 300
Ouabain (23,900 ng/ml)	2010 ± 60
Dexamethasone (308 ng/ml) + ouabain (23,900 ng/ml)	1930 ± 30

dependence of AIB uptake on the concentration of AIB was similar to that observed previously (17), with an apparent K_m for AIB uptake of about 1 mM (Fig. 5). In the presence of prednisolone the apparent K_m of the cells for AIB decreased, without appreciable increase in the V_{max} of transport (Fig. 5).

Effect of steroids on cell viability and DNA synthesis. At the concentrations and over the time periods employed, neither prednisone nor prednisolone caused the cells to lose their ability to exclude a vital dye (trypan blue) or to lose their viable appearance under phase-contrast microscopy. Because previous experiments with mouse 3T3 fibroblasts have demonstrated that glucocorticoids initiate proliferation (8, 9), the effect of prednisone and prednisolone on thymidine incorporation was evaluated in the present series of experiments (Table 5). Whereas prednisone (78 ng/ml) was without effect on the baseline incorporation of [^{3}H]thymidine into acid-insoluble material, prednisolone (78 ng/ml) significantly reduced baseline thymidine incorporation. Additionally, prednisolone diminished appreciably, but by no means totally, the cellular response to a specific human fibroblast mitogen, epidermal growth factor (17, 18, 20); prednisone was ineffective in decreasing epidermal

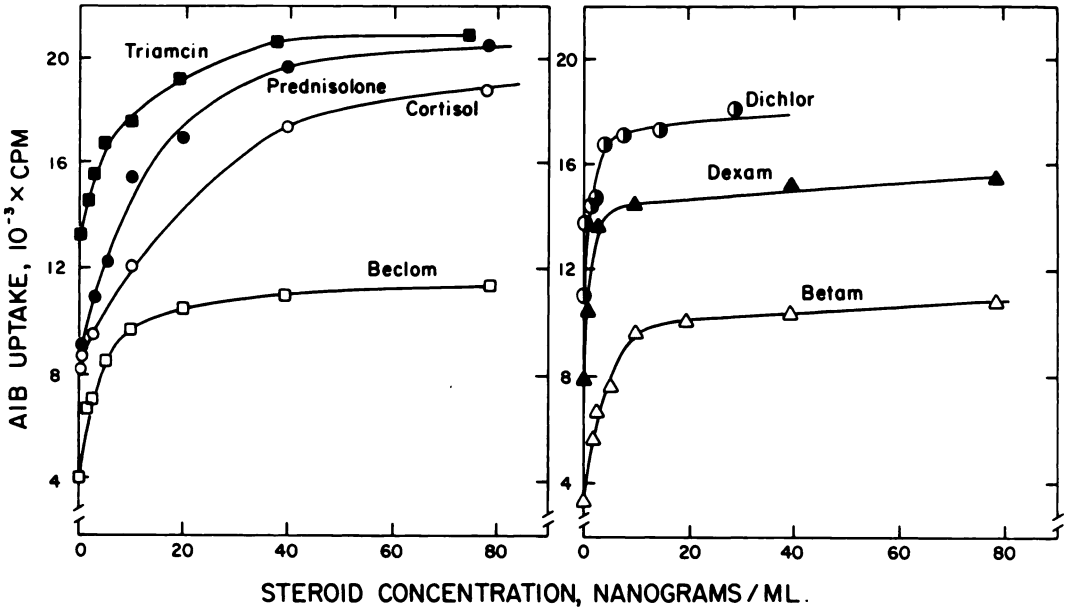


FIG. 4. *Dose-response curves for steroid-stimulated AIB uptake*

Monolayers were treated as described in the legend to Fig. 1 with increasing amounts of the indicated glucocorticoids. After the final change of buffer, the uptake of AIB (7.4 μM, final concentration; 400 cpm/pmole) during a 12-min period was measured as described in MATERIALS AND METHODS. Values represent the mean uptake per monolayer for three replicate measurements. ○——○, cortisol; ●——●, prednisolone; □——□, beclomethasone; ■——■, triamcinolone; △——△, betamethasone; ▲——▲, dexamethasone; ◑——◑, dichlorisone.

TABLE 4

Potencies and structural features of glucocorticoids that stimulate AIB uptake

Values for half-maximal stimulation of AIB uptake (ED_{50}), representing the mean ± standard deviation for the number of determinations indicated in parentheses, were estimated from independently measured dose-response curves as exemplified in Fig. 4. The replacement dose of steroid reflects the clinically observed glucocorticoid and anti-inflammatory activity in humans (26).

Compound	ED_{50} for AIB uptake			Daily oral replacement dose		Substitution in cortisol structure at position(s)			
						1,2	11	9	16
	ng/ml		*M* × 10^8	*mg*	*moles* × 10^5				
Cortisol	16	± 3.7 (4)	4.4	20	5.5	None	None	None	None
Prednisolone	7.9	± 1.5 (4)	2.2	4	1.1	Δ[a]	None	None	None
Triamcinolone	5.1	± 2.7 (2)	1.3	5	1.3	Δ	None	α-F	α-OH
Beclomethasone	2.9	± 0.9 (3)	0.7	ND[b]	ND	Δ	None	α-Cl	β-CH_3
Betamethasone	2.7	± 0.2 (2)	0.7	0.6	0.15	Δ	None	α-F	β-CH_3
Dichlorisone	1.6	± 0.5 (3)	0.4	ND	ND	Δ	β-Cl	α-Cl	None
Dexamethasone	0.75	± 0.07 (2)	0.2	0.75	0.19	Δ	None	α-F	α-CH_3

[a] Δ = double bond.

[b] Not determined.

growth factor-stimulated incorporation of thymidine. It can be concluded that the cells remain essentially viable and responsive to a mitogen in the presence of steroid, and that the steroid-mediated increases in AIB uptake are not consequent to an intrinsic mitogenic action of the glucocorticoids.

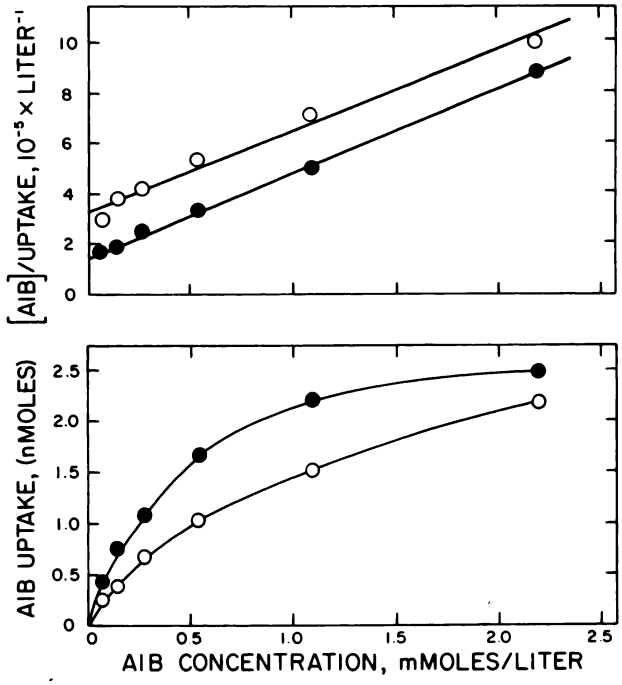


FIG. 5. *Concentration dependence of AIB uptake in fibroblast monolayers*

Monolayers with (●——●) or without (○——○) prednisolone (78 ng/ml) were treated as described in the legend to Fig. 1. After the final change of buffer, the uptake per monolayer during a 12-min period was determined for increasing concentrations of AIB. The lower figure shows the uptake of AIB per monolayer; the upper figure shows a plot of the data according to Hanes (27). The data from the experiment indicate a steroid-mediated reduction in the apparent K_m for AIB from 1.0 to 0.43 mM; the maximum uptake rate for both treated and untreated monolayers was 3 nmoles/12 min/monolayer.

DISCUSSION

The present study reveals that functional receptors for glucocorticoids can be readily evaluated in explant skin-derived human fibroblasts. An encouragingly close parallel is observed between the relative potencies for stimulating AIB uptake in the cultured cells and the clinical ranking of the same steroids for their glucocorticoid and anti-inflammatory activities in humans. The study compliments independent measurements of glucocorticoid binding to cytosol receptors from cultured human fibroblasts (11) and provides an independent approach to the study of glucocorticoid action in a human-derived tissue. The assay of biological responsiveness provides certain advantages over studies of ligand binding, in that the relative receptor affinities may be evaluated more precisely and conveniently. In addition, there are serious technical limitations to the rigorous study of the affinities of hormones for receptors with K_D values in the 10^{-8} M range (e.g., very rapid dissociation, effects of receptor and ligand concentrations on estimates of potencies from binding competition studies); these problems have been considered at length in connection with studies of polypeptide hormone receptors (15). Despite these reservations, it is important to note (11) that in binding competition studies, the relative ability of glucocorticoids to compete for the binding of ^{3}H-labeled triamcinolone acetonide to fibroblast cytosol receptors was dexamethasone > prednisolone > cortisol. The same rank order is observed in the present study for the biological action of these compounds in similar cultured fibroblasts, and it is tempting to attribute differences in biological potency to differences in intrinsic receptor affinities rather than to differences in the metabolism of the steroids.

TABLE 5

Inhibition of thymidine incorporation by prednisolone

Reagents at the indicated final concentrations were added to confluent monolayers 5 days after the final change of growth medium. Twenty-four hours after the addition of reagents, the incorporation of [^{3}H]thymidine (1 μCi/ml; 6.7 Ci/mmole) into acid-insoluble material was measured after a further 2-hr incubation at 37°. Results are means ± standard deviations.

Additions	Thymidine incorporation
	cpm
None	800 ± 90
Prednisolone (78 ng/ml)	340 ± 30
Prednisone (78 ng/ml)	760 ± 50
EG factor[a] (14 ng/ml)	6730 ± 210
EG factor (14 ng/ml) + prednisolone (78 ng/ml)	5290 ± 100
EG factor (14 ng/ml) + prednisone (78 ng/ml)	6510 ± 190

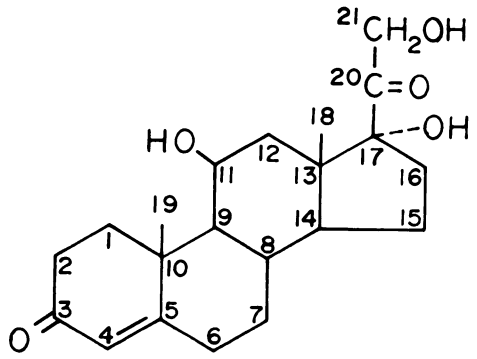
[a] Epidermal growth factor.

It may be an advantage to assess the functional potency of glucocorticoids in human-derived material, rather than with the use of cells such as rat hepatoma tissue culture cells (5, 28) or tissues from other species. It is evident that, while the rank order of potencies of compounds in different species may not vary, aboslute potencies may differ from species to species (26). In particular, the present study indicates a much higher intrinsic glucocorticoid activity of the 21-alcohol form of the chlorinated steroids, dichlorisone and beclomethasone, than might have been predicted from other measurements *in vivo* in the rat (29, 30). Of particular interest is the high potency of dichlorisone in the cultured cells, which further emphasizes the novel observations by Robinson and co-workers (31) and by Glenn *et al.* (32) that 11-oxygenation is not a prerequisite for potent anti-inflammatory activity.

It was somewhat surprising that the 11-keto analogues (prednisone, 16β-methylprednisone, cortisone) were inactive in stimulating AIB uptake even after 17 hr of incubation with the monolayers, since some enzymatic interconversion (11-keto to 11-hydroxy) was anticipated. There is, however, precedent for the lack of such interconversion in studies with cultured cells (7, 8), and it may be concluded that, in the present study with human fibroblasts, the interconversion did not appreciably affect the measurements. Further work is being done to analyze the metabolism of glucocorticoids in fibroblast monolayers, so as to explore one possible explanation for the lack of response to steroids, but not to insulin, of some fibroblast monolayers in the present study.

The steroid-induced changes in AIB uptake observed for human fibroblasts in the present study most closely parallel analogous observations made in the isolated, perfused rat liver, where both hydrocortisone and insulin increase the uptake of AIB in an additive manner (33). In contrast, in cultured rat hepatoma cells, dexamethasone reduces the uptake of AIB, whereas insulin stimulates AIB uptake in both the presence and absence of dexamethasone (5). The basis for these differences in response cannot be readily explained at present.

It is well recognized that the parameter of cellular responsiveness monitored in the present study (AIB uptake) is modulated by a number of factors (e.g., pH, sodium gradient, protein synthesis). It may therefore be difficult to ascertain the precise molecular event related to amino acid uptake which is affected by glucocorticoids. The change in AIB uptake appears to be an event "distant" from the initial hormone-receptor interaction, as indicated by the prolonged time course of action and the sensitivity of steroid-stimulated AIB uptake to ouabain, cycloheximide, and (to a lesser extent) puromycin. This steroid effect on transport is to be contrasted with the apparently immediate effect of insulin on the rat fat cell membrane transport system for 3-*O*-methyl-D-glucose (34–37). By the use of fibroblast cultures in the manner indicated here it should be possible not only to examine more closely the hormonal modulation of membrane transport in general but to determine whether hormones control the synthesis of specific components of a discrete amino acid transport system; AIB uptake most likely reflects the activity of the "A system" for the



Cortisol

transport of naturally occurring amino acids, originally characterized in the Ehrlich cell (38).

The present study provides a basis for the functional evaluation of the intrinsic potency of glucocorticoid compounds in humans and furnishes evidence for a high intrinsic potency of the 21-alcohol chlorinated glucocorticoids, beclomethasone and dichlorisone. Additionally, a basis is provided for the study of the hormonal modulation of amino acid transport in an accessible human tissue in a manner not easily performed *in vivo*. Human fibroblast cultures may therefore prove of value for the preliminary evaluation of new glucocorticoids for use in humans.

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