

Synthetic glucocorticoids potentiate IgE synthesis

Influence of steroid and nonsteroid hormones on human *in vitro* IgE secretion

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Recently, hydrocortisone (HC) has been shown significantly to enhance interleukin-4 (IL-4)-induced *in vitro* IgE synthesis. For investigation of possible effects of synthetic corticosteroids but also of effects of other important human hormones, peripheral blood mononuclear cells (PBMC) were incubated with IL-4 and various concentrations of the hormones. IgE secreted in the supernatant was determined after a 14-d culture period. Like HC, all synthetic corticosteroids potentiated IgE secretion. A minor effect was noted for the mineralocorticoid aldosterone. No modulating effect on IL-4-induced IgE formation was observed for adrenocorticotrophic hormone (ACTH), somatotropin (STH), thyroid-stimulating hormone (TSH), triiodothyronine, thyroxine, epinephrine, noradrenaline, insulin, and glucagon.

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Recently, it has been shown that interleukin-4 (IL-4) induces human peripheral blood mononuclear cells (PBMC) to secrete IgE (7, 10, 27, 30, 39). To produce IgE, human B cells need to receive at least one further signal besides IL-4. This signal is normally delivered by T cells (9, 10, 26, 29, 36). Recent reports indicate a major role for the interaction of CD40 and its corresponding ligand on the T cell for this antigen-nonspecific, contact-dependent T-B cell interaction (16, 17, 24). The T-cell signal is not necessary when B cells are costimulated with hydrocortisone (HC) (29, 38). HC not only removes the requirement for T cells in human *in vitro* IL-4-induced IgE synthesis by B cells, but also strongly augments IL-4-induced IgE secretion in unseparated PBMC (13, 29, 38). The HC effect is probably not mediated via the CD40 pathway because the stimuli by HC and the T cells act synergistically on the synthesis of IgE (29, 38).

Because of their anti-inflammatory and immunosuppressive effects, synthetic glucocorticosteroids are widely used in the treatment of various disorders, including the control of IgE-mediated diseases.

The present study investigated the effect of commonly applied synthetic cortisone derivatives on

human IL-4-induced IgE synthesis. Since HC has such a striking effect on IgE formation, we also studied possible influences of other hormones.

Material and methods

Reagents

Recombinant IL-4 isolated from supernatants of transfected Chinese hamster ovary cells was kindly provided by Dr H. P. Kocher (Sandoz, Basel, Switzerland). HC, prednisone, prednisolone, 6- α -methyl-prednisolone, triamcinolone, fludrocortisone, dexamethasone, betamethasone, aldosterone, triiodothyronine thyroxine, TSH, epinephrine, noradrenaline, ACTH, STH, and glucagon were purchased from Sigma (Deisenhofen, Germany). Recombinant human insulin was obtained from Hoechst (Frankfurt, Germany).

Cell cultures

Heparinized venous blood was obtained from normal blood donors with no history of allergic diseases, and IgE serum levels of < 100 ng/ml. PBMC

were isolated by Ficoll/Hypaque density gradient centrifugation. They were washed three times in RPMI 1640 supplemented with 2 mmol/l glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B ("medium"), and 2.5% fetal bovine serum (Gibco Paisley, UK).

The cells were cultured in medium containing 10% fetal bovine serum in 96-well, round-bottom culture plates (Nunc, Copenhagen, Denmark) in a humidified atmosphere of 5% CO₂ and 95% air. PBMC were used at a concentration of 2×10^6 cells/ml. IL-4 was added at 200 U/ml. Corticosteroids, aldosterone, and thyroxine were dissolved in DMSO, epinephrine and noradrenaline in 3.5×10^{-2} M HCL, insulin in 10^{-1} M HCL, and triiodothyronine in 10^{-2} M NaOH, according to the manufacturer's instructions. Glucagon, ACTH, STH, and TSH were directly dissolved in culture medium. The highly concentrated stock solution was then further diluted in medium, as indicated in the Results. All cultures were done in five identical replicates, and the supernatants were collected after 14 d. The release of cell-associated immunoglobulin performed *in vivo* was determined in cultures containing 100 µg cycloheximide/ml (Sigma). All data in the Results are given as IgE net synthesis, calculated by the subtraction of preformed and spontaneously secreted immunoglobulin.

IgE measurement

IgE secreted into the cell supernatants was measured by an enzyme-linked immunosorbent assay, which has previously been described in detail (28): 96-well plates (Nunc) were incubated with a rabbit-antihuman-IgE (Behring) for 1 h, and then washed three times with buffer; subsequently, the nonspecific binding sites were blocked with a 10% FCS-buffer solution for 30 min. Dilutions of the culture supernatants, or purified myeloma protein standards (kindly provided by Dr H. L. Spiegelberg), and a supernatant pool of known IgE content were added and incubated for 1 h. After washing, the plates were incubated with a biotinylated goat-antihuman-IgE (Medac, Hamburg, Germany) for 1 h. An additional washing step was followed by the addition of the streptavidin/peroxidase-complex (Amersham Buchler, Braunschweig, Germany) for 30 min. The plates were washed again, and, finally, the substrate *o*-phenyldiamine-dihydrochloride (Sigma) and H₂O₂ were added. The staining reaction was stopped after 15 min with 2 M HCL. The optical density was measured at 492 nm and 630 nm. The IgE concentrations were calculated from appropriate standard curves. Mean values of the replicate cultures were calculated.

Results

Effect of hydrocortisone

As shown in Fig. 1, HC potentiates IL-4-induced IgE production by PBMC from normal blood donors in a dose-dependent manner. Even a concentration of 10^{-7} M HC can increase IgE synthesis about 20-fold, whereas highly concentrated HC (10^{-3} M) failed to enhance IgE secretion. This is probably due to the toxic concentration of the solvent DMSO, which is necessary to prepare 10^{-3} M HC. The dose-response curve also covers the physiologic cortisone serum level of about 10^{-6} M. The addition of HC alone without IL-4 did not induce substantial IgE synthesis over the spontaneous production by PBMC (data not shown).

Effect of synthetic corticosteroids

For the treatment of various disorders, including allergic diseases, a large panel of synthetic glucocorticoids has been developed. Like HC, these drugs can also strongly increase IL-4-induced IgE synthesis. The effect of a selection of widely applied corticosteroids is shown in Fig. 1. According to the anti-inflammatory potential relative to HC, which is about fourfold for prednisone and prednisolone, fivefold for triamcinolone and fludrocortisone, and 30-fold for betamethasone and dexamethasone, a shift in the dose-response relation could be observed. While HC is not active at concentrations of $<10^{-7}$ M, the very potent corticosteroids, in particular, are able to augment IgE production even at a concentration of 10^{-9} M. Like HC, highly concentrated hormones (10^{-3} M) did not coinduce IgE synthesis. Moreover, PBMC were not stimulated to secrete IgE by the hormones alone (data not shown).

Effect of aldosterone

For investigation of a possible influence of mineralocorticosteroids, aldosterone was tested. As shown in Fig. 2, aldosterone also increased IgE synthesis. However, this effect was quantitatively minor, as compared with glucocorticosteroid-induced IgE formation, and could be observed only in a dose range of 10^{-4} – 10^{-6} M aldosterone. This is, at least, about 1000 times the amount found in the serum under physiologic conditions.

Effect of other hormones

As in the experiments with the steroid hormones, the effect of other nonsteroid hormones on IL-4-induced IgE synthesis was also tested. Table 1 summarizes the results of a large number of experiments with

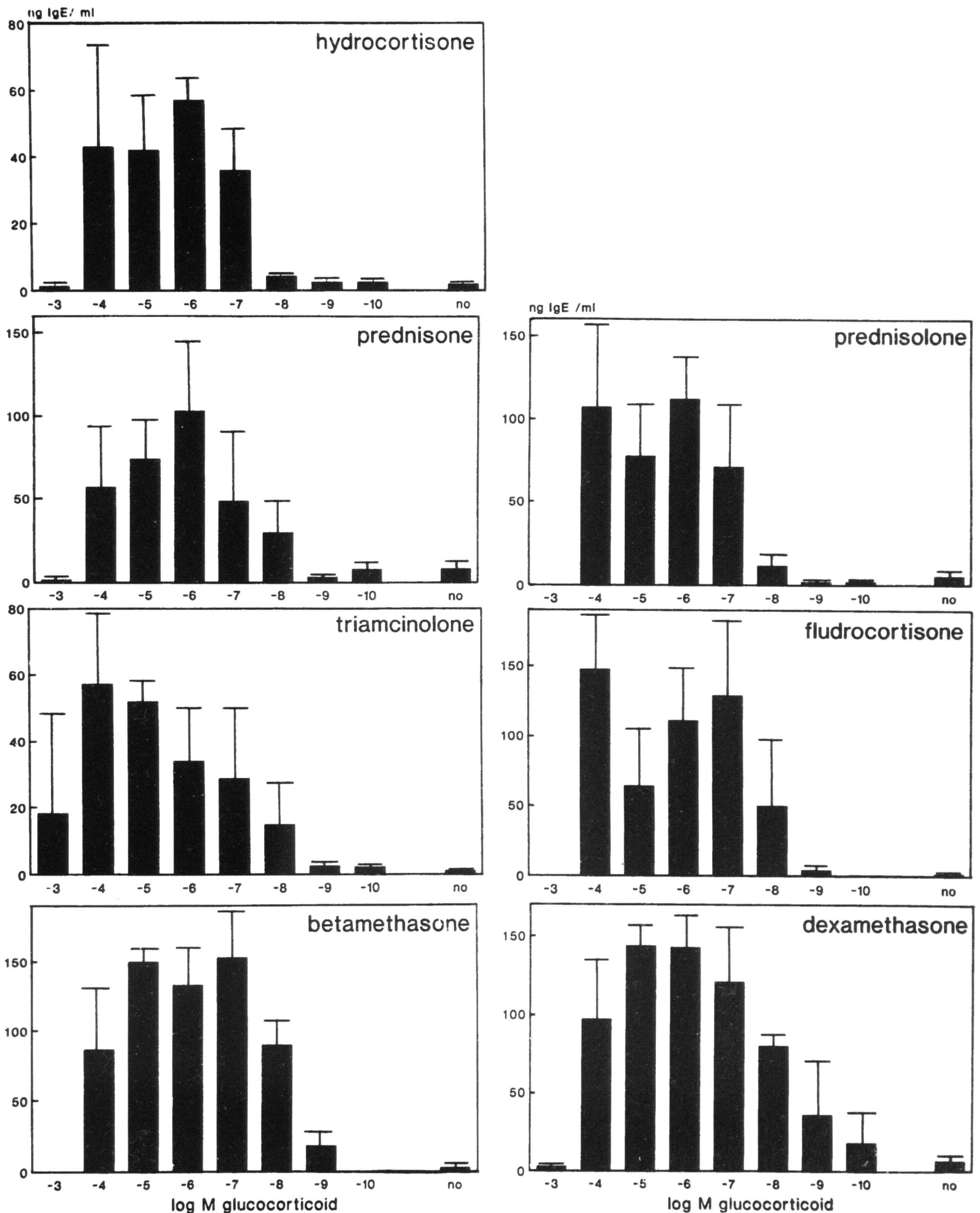


Fig. 1. Dose-response curves of effect of HC and of various synthetic glucocorticoids on IL-4-induced formation of IgE. PBMC were used at 2×10^6 cells/ml. All cultures received 200 U IL-4/ml. For results on HC, mean $\pm$ SEM from five different experiments with different donors are given. For synthetic glucocorticoids, each experiment depicted shows representative curve from at least three different experiments performed with each hormone yielding similar results (mean values $\pm$ SD).

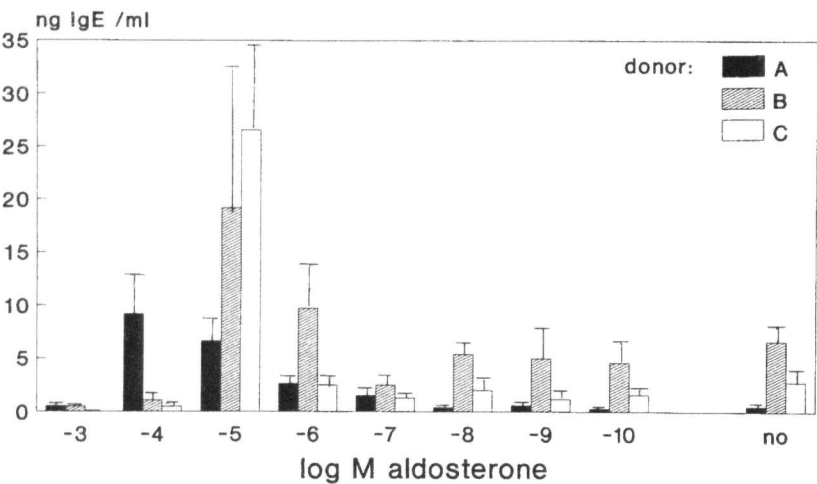


Fig. 2. Effect of aldosterone on IL-4-induced IgE production. PBMC were used at 2×10^6 cells/ml. All cultures received 200 U IL-4/ml. Dose-response curves of three different experiments with three different donors are shown (mean values $\pm$ SD).

various hormones. There was no significant effect on IgE synthesis by any of the hormones tested.

Discussion

Specific intracytoplasmic corticosteroid receptors have been identified in human lymphocytes (25), and HC has been shown to induce profound and complex effects on the human immune response (8). Particularly, it can strongly enhance IgG, IgA, and IgM production by B cells (14). Recently, its enormous potential to increase human IL-4-induced IgE secretion has been discovered (13, 29, 38).

Our data show that HC can potentiate IL-4-mediated IgE secretion by PBMC within a wide range of concentrations. Interestingly, this also includes the physiologic serum level for HC of about 10^{-6} M. This implies that, provided the *in vitro* results can be readily transferred to the *in vivo* situation, HC is an important and essential factor for normal human *in vivo* IgE synthesis. In mice, the height of *in vivo* IgE synthesis has been found to be temporally related to diurnal variation in endogenous steroid production (2).

All synthetic cortisone derivatives are equally effective, indicating that binding to the HC receptor is crucial for their IgE-enhancing effect. This underlines the observation that synthetic steroids, designed to have an increased anti-inflammatory potential, show a dose-response shift paralleling their known intrinsic receptor activity. Moreover, a specific receptor antagonist can block the IgE-potentiating effect of HC (38).

From a clinical point of view, these results are disturbing, because the synthetic glucocorticoids are widely applied, particularly for the treatment of allergic disorders. Therefore, if this IgE-potentiating effect is also true for the *in vivo* situation, glucocorticoid therapy would entail the risk of an increased IgE synthesis and even the risk of new sensitizations. There are only a few older studies dealing with the role of glucosteroid treatment on IgE serum levels of allergic patients (15, 18, 31, 33). Whereas two groups reported an increase of IgE during steroid treatment (31, 33), two others did not observe significant changes in serum levels (15, 18). Because of the new *in vitro* evidence for the IgE-potentiating ability of corticosteroids and the clinical relevance of the question of whether cortisone treatment even increases the allergic risk, we are currently investigating the effect of oral prednisone in a therapeutic dose on IgE serum levels of normal subjects.

The mineralocorticosteroid aldosterone is known to have a small corticotropic effect with regard to carbohydrate metabolism, but it has no measurable anti-inflammatory potential. In our experiments, aldosterone had a small but significant influence on IL-4-induced IgE formation. Since this effect is observed at aldosterone concentrations about 1000 times higher than physiologic concentrations, it is of little clinical relevance. Other steroid hormones such as the sexual hormones testosterone, β -estradiol, and progesterone have already been shown not to affect IL-4-stimulated IgE synthesis (38).

Neuroendocrine peptides have been reported to have lymphokine-like properties and modulate im-

Table 1. Nonsteroid hormones unable to modulate IL-4-induced IgE secretion by PBMC*

Hormone	Physiologic serum concentration	Concentration range tested	Effect on IgE synthesis
ACTH	$1-7 \times 10^{-5}$ μ g/ml	$10^{-6}-10$ μ g/ml	none
STH	$10^{-7}-10^{-8}$ U/ml	$10^{-8}-10^{-1}$ U/ml	none
TSH	$10^{-7}-10^{-5}$ U/ml	$10^{-7}-10^{-3}$ U/ml	none
Triiodothyronine	$0.5-2 \times 10^{-3}$ μ g/ml	$10^{-7}-1$ μ g/ml	none
Thyroxine	$0.4-1.2 \times 10^{-2}$ μ g/ml	$10^{-7}-1$ μ g/ml	none
Epinephrine	$0.7-2.5 \times 10^{-4}$ μ g/ml	$10^{-7}-1$ μ g/ml	none
Noradrenaline	$3.1-4 \times 10^{-4}$ μ g/ml	$10^{-7}-1$ μ g/ml	none
Insulin	$10^{-6}-10^{-5}$ U/ml	$10^{-7}-1$ U/ml	none
Glucagon	$10^{-4}-10^{-3}$ μ g/ml	$10^{-7}-1$ μ g/ml	none

* 2×10^6 PBMC/ml were incubated with 200 U IL-4/ml and various concentrations of respective hormone. For each hormone, at least three different experiments with different donors were performed.

mune responses (3, 37). High-affinity receptors for ACTH and STH have been detected on human lymphocytes (5, 21, 34). ACTH is known to increase B-cell proliferation (1, 6), but to suppress antibody formation (19, 20, 37), and it also decreases INF- γ production by T cells (19, 20). STH modulates the proliferation of T cells and their differentiation into cytotoxic T cells (35, 37). TSH has been shown to increase both B-cell proliferation and immunoglobulin formation (4, 22). Dwarf mice with hypopituitarism and therefore defective STH and TSH production have a deficient immune system (11). Since this immunodeficiency can be overcome by injections of STH, TSH, and thyroxine (12), an influence on the immune system by thyroid gland hormones is likely. Despite all those known pleiotropic effects on the immune system, these hormones did not modulate IgE synthesis by PBMC either alone or together with IL-4.

We also failed to demonstrate an effect of the catecholamines epinephrine and noradrenaline, which are, like HC, released in stress situations, or of the glucose-metabolism-regulating hormones insulin and glucagon. Whereas little is known about catecholamine effects on the immune system, insulin, in particular, has been reported to influence other B-cell functions (22, 32).

Taken together, our experiments show that corticosteroids significantly enhance, whereas other major human hormone systems do not affect, IL-4-induced IgE formation *in vitro*. Because of its clinical importance the *in vivo* effect of synthetic glucocorticoids on IgE synthesis has to be clarified.

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