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Prognostic Value of Steroid Receptor Determination in Leukemia[1]

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Abstract

Determinations of steroid receptors have been used to predict steroid sensitivity in various neoplastic tumors. It appears, however, that simple steroid binding measurements are not sufficient for that purpose in lymphoid tumors. This conclusion is based on a literature survey showing, first, that numerous factors are capable of modulating cellular steroid receptor content; second, that the results of steroid receptor determinations are critically dependent on experimental procedures; and, third, that the correlation between steroid receptor content and sensitivity is not obligatory in animal or human leukemic cells.

Introduction

In all steroid-responsive tissues the initiation of hormonal response appears to be mediated by a specific receptor system in the cytoplasm of the target cells. The main argument supporting this assumption is the close correlation between the affinity of various steroids for the cytoplasmic binding sites and physiological potency (3, 10, 16, 19, 65).

Given the fact that the binding step is an obligatory event in the mechanism of steroid action, attempts have been made to infer the steroid sensitivity of various tissues from the determination of their steroid receptor content. In particular, this procedure has been used in neoplastic tissues such as mammary tumors, prostatic carcinoma, or leukemia. Recent reports, however, suggest that in lymphoid tissue it is not possible to correlate properly cytoplasmic receptor levels with glucocorticoid responses (6, 32, 45).

Therefore, we want to present a critical reevaluation of receptor determination as an index of steroid sensitivity in lymphoid tissue.

Physiological Modulation of Steroid Receptors

Milgrom *et al.* (50) have emphasized the possibility that the hormonal control of target cells could depend not only on variations in the plasma levels of steroids but also on changes in the cellular amount of receptors. They demonstrated that the level of progesterone receptors undergoes an alternative increase and diminution during the estrous cycle and also rises after a single injection of estradiol. Similarly, estradiol in the uterus appears to be able to modulate its own receptors. An injection of estradiol in immature rats induces an initial drop in estradiol receptors followed by a low replenishment to control and even higher values.

In the case of glucocorticoids, adrenal ablation produces an increase in corticosterone binding sites in rat liver (4, 18) and heart (26). We have recently demonstrated that adrenalectomy in mice produces not only an increase in thymus size and cellularity but also an augmentation of the number of dexamethasone binding sites per cel[1].[2] This elevation of receptors appears to be due to a true increase in the number of receptors rather than to an unmasking of sites previously occupied by endogenous hormone (26).[2] The existence of a possible mechanism of receptor regulation raises the question of such a modulation under conditions when fluctuations of steroid plasma levels are observed, such as circadian rhythms, stress, and even steroid therapy.

Evaluation of receptor amounts in target organs is made on the basis of protein or DNA content or per cell, assuming that the binding sites are uniformly distributed. This assumption, however, could be inaccurate, particularly in the lymphoid tissue where different cell subsets with different origins and different sensitivities to steroids have been characterized (2, 9, 74). Attempts to isolate subpopulations and to measure their steroid receptors have given rise to puzzling results. Schaumburg and Crone (67) showed that in chickens thymus and bursa lymphocytes contain different amounts of glucocorticoid receptors (600 and 1200 sites/cell, respectively). In mice, spleen cell subpopulations rich in B- and T-cells do not bind the same amount of steroids,[3] whereas thymocyte subfractions contain similar quantities of binding sites (14). In humans no difference in receptors could be demonstrated between subpopulations of circulating lymphocytes (42).[4] Examination of thymus cells in children, however, reveals a low number of glucocorticoid receptors as compared to circulating lymphocytes in adults.[5] Moreover, the hormonal state could deeply influence the repartition of the lymphocyte subpopulations. Shortman and Jackson (69) showed that adrenalectomy or hydrocortisone treatment modulates the percentage of high θ and low θ thymus cells in mouse. Yu *et al.* (79) and Fauci and Dale (17) demonstrated that steroid treatments in humans induce changes in lymphocyte recirculation and homing. Therefore, determination of the steroid receptors should take into account the species, the nature of the subpopulation studied, and the hormonal state of the individual.

Claman (9) and Baxter *et al.* (2) emphasized that in

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lymphoid tissue glucocorticoid susceptibility depends on not only the immunological origin of the cell but also on parameters such as the degree of maturation or differentiation and the stage of cell proliferation. Several authors have attempted to follow the ontogeny of glucocorticoid receptors in parallel with the onset of glucocorticoid-inducible functions in embryos and neonates.

According to the organs studied, several patterns of development could be observed. In chick neural retina Koehler and Moscona (37) described a decrease of hydrocortisone receptors during embryonic life, whereas Lippman *et al.* (47) were unable to demonstrate receptor changes during the same period. Similar discrepancies exist for steroid receptor development in rat liver between the results of Feldman (18) and those of Giannopoulos (23). Giannopoulos *et al.* (24) reported a 2-fold decrease of thymus binding sites in rabbits during the neonatal period. However, we recently showed that in mice no changes in steroid receptors occur in spleen or thymus during the neonatal period.[2] Because of these conflicting results, the pattern of ontogeny of steroid receptors remains essentially obscure and requires additional investigations. Possible changes of steroid binding sites during aging have received little attention, but Roth (63) and Roth and Livingston (64) have described a decrease of the receptor content in both leukocytes and adipocytes with senescence. In addition, in a given cell the level of binding sites fluctuates according to the stage of cell proliferation. Cidlowski and Michaels (7) demonstrated in synchronized HeLa cells that the number of glucocorticoid receptors increases during S phase and falls after mitosis. Similarly, mitogen-induced blast transformation of human lymphocytes is associated with an elevation of the receptor content/cell (53, 71).

Finally, the extent of steroid binding to the receptors could be dependent on metabolic parameters or modulated by other factors. Munck and Brinck-Johnsen (51) and Munck *et al.* (52) showed that the binding of cortisol to its cytosolic receptors was virtually abolished in the absence of glucose or under anaerobic conditions. Nielsen *et al.* (54) pointed out that dephosphorylation decreases receptor binding capacity, suggesting that the binding process could be energy dependent. Several authors postulated the existence of factors capable of modulating steroid receptor interactions; these factors include phospholipids (68), steroids (72, 75), and unidentified modulators (11, 12, 55, 66).

The different parameters responsible for *in vivo* and perhaps physiological modulations of steroid binding sites are summarized in Table 1.

Table 1
Modulation of cellular steroid receptor content

Parameter	Conditions	Refs.
Level of circulating steroid	Steroid injection	50
	Adrenalectomy	4, 18, 26
	Stress	?
	Circadian rhythm	?
	Hormonal therapy	?
Nature of cell subpopulation	Immunological origin	13, 14, 38, 42, 67
	Changes of repartition with hormonal state	17, 69, 79
Cell differentiation	Ontogenesis	18, 23, 24, 37, 47
	Aging	63, 64
	Cell cycle	7
	Blast transformation	53, 71
Metabolic conditions		51, 52, 54
Other factors	Phospholipids	68
	Steroids	72, 75
	Unknown modulators	11, 12, 55, 66

Conditions of Steroid Receptor Determination *in Vitro*

Some of the discrepancies in the results of different groups working on the same material can be attributed to the methods of binding site measurement. Indeed, quantitative evaluation of steroid receptors appears to be critically dependent on experimental parameters such as isolation of subcellular fractions, temperature, composition of the incubation medium, and nature of the tracer. In lymphoid tissue it is possible to determine steroid receptors either in cell-free cytosolic extracts of 0–4° or in whole-cell assays at 37°. The characteristics of dexamethasone binding in human circulating lymphocytes could therefore differ by 1 order of magnitude according to the method used (31, 42). Moreover, in the whole-cell procedure at 4° and 37° we demonstrated that glucocorticoid receptor specificity varies with temperature (15). In contrast to estrogen and androgen receptors, glucocorticoid receptors appear extremely unstable, Kaine *et al.* (34) showed that, during the preparation of thymus cytosol by centrifugation, 20 to 30% of the steroid binding capacity could be lost. Given the fragility of these binding proteins, numerous parameters are capable of affecting their interaction with the ligand, either stabilizing or accelerating receptor decline.

These factors include glycerol, saccharose, cations, EDTA, pH, ionic strength, steroids, and enzymes (34, 39, 59, 68, 72, 75). Sulfhydryl groups appear to play a key role in the binding process, as recently illustrated by Granberg and Ballard (25).

The nature of the tracer used in receptor determination might modify the results. First, triamcinolone acetonide and dexamethasone are more tightly bound by glucocorticoid receptors than are natural steroids (*e.g.*, corticosterone, cortisol) (5, 15). The use of synthetic molecules thus decreases the possibility of steroid receptor dissociation during the experimental procedure. Second, corticosterone or cortisol is known to interact with plasma proteins or with corticosteroid-binding globulin-like binders in the cytoplasm of target tissue (20, 21, 48), therefore leading to a possible overestimation of receptor sites. The existence of a corticosteroid-binding globulin-like protein in human lymphocytes has been reported by Werthamer *et al.* (78) but could not be demonstrated in mouse thymus (15).

Animals used as experimental material are usually adrenalectomized to avoid competition between the endogenous hormone and the exogenous tracer for binding sites. However, in humans the presence of endogenous steroid filling part of the binding sites could lead to an underestimation of the receptors. Until now, attempts to draw an exchange assay between nonradioactive and radioactive

ligands to measure the totality of the cytosolic glucocorticoid receptors have been unsuccessful (18, 26). Therefore, in humans the actual determinations appear to be only estimations of the empty sites. We recently obtained evidence suggesting that an exchange assay could be performed in the whole-cell assay procedure.[2]

In addition, it was surprising that treatment of intact cells with agents that produce membrane alterations (neuraminidase, phospholipase) or modify the properties of the membrane (concanavalin A, iodoacetamide) induces a diminution of steroid receptor levels (8, 27–29, 49, 58).

Relationship between Steroid Sensitivity and Glucocorticoid Receptor Levels in Normal and Neoplastic Lymphoid Cells

Suggestions that steroid sensitivity could be correlated with the levels of cellular glucocorticoid receptors arose essentially from experiments performed on neoplastic cell lines *in vitro*. Numerous workers reported a defect of steroid receptors in resistant clones as compared to their steroid-sensitive counterpart. In S_{49}IA lymphoma cells or in P1798 lymphosarcoma cells, for example, resistant cells contain 30 to 50% of the binding sites measured in sensitive ones (2, 30, 33, 35, 36, 62, 76). In contrast to these tissue cultures, where steroid sensitivity is easily appreciated following cell lysis, growth, or differentiation inhibitions, steroid susceptibility is more difficult to determine in human leukemia. Usually, antileukemic therapy includes steroid (prednisolone) and other cytotoxic drugs. It is therefore difficult to ascertain the efficiency of one among these agents. Moreover some patients who initially respond to steroid therapy do not respond at a later date, suggesting that cells initially sensitive become resistant or are replaced by resistant cells (35, 36). Despite these difficulties Lippman *et al.* (43, 44, 46, 47) presented evidence that clinical sensitivity to steroids could be associated with elevated levels of steroid binding sites in peripheral cells from patients with acute lymphoblastic leukemia or acute myeloblastic leukemia.

This fascinating correlation between receptor content and steroid sensitivity has recently been questioned, particularly by Sibley and Tompkins (70). These authors developed corticoresistant variants from the S_{49}IA-sensitive cell line. Among these variants the majority are defective in receptors, but some of them contain receptors indistinguishable from those of the sensitive control.

From the experiments performed in various systems, several points become apparent. First, neoplastic tissues often contain the same receptor contents as normal tissues (41). Second, elevation of receptor content does not induce steroid hypersensitivity (71), and, third, glucocorticoid resistance is not always associated with receptor defect (13, 14, 40, 73).

Furthermore, attempts to associate the steroid binding capacity and the clinical state in human neoplastic lymphoid tissue have given only moderate (22) or negative results (6, 31, 32, 77).[6] This situation is somewhat comparable to that occurring in breast tumors, in which all tumors that contain estrogen-binding proteins do not necessarily respond to additive or ablative hormone therapy. The conclusion is that, although receptors appear to be indispensable for sensitivity, sensitivity is not governed by receptors alone but depends on many other steps in the mechanism of action of glucocorticoids. Therefore, receptor determination in human leukemic cells is not sufficient to preclude steroid sensitivity.

[6] G. Crabtree, K. Smith, and A. Munck, submitted for publication.

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