

Factors Affecting Adrenocortical Hormone Function

by E. Brad Thompson*

This paper will briefly outline those elements which must be considered in assessing the effects of any given compound which might impinge on the actions of adrenocortical steroids. Considerations that ought to be taken into account include the following: the delivery to the affected cells of corticosteroids, the uptake of the hormone by the cells, the metabolism of the hormone by the cells, the intracellular actions of the hormone, the possible secondary interactions between cells, and multihormonal actions on the final target cell.

Delivery of Corticosteroids to Target Tissues

The astute reader will already have noted that by beginning with the question of hormone delivery, I have neglected synthesis and its regulation. I have done so because synthesis seems outside the topic of factors affecting corticosteroid function, though of course the ultimate behavior of hormone-sensitive tissues depends on accurate control of hormone synthesis. For a consideration of this important and complex topic, therefore, the reader is referred to standard works on the subject. The most important concept regarding circulating corticosteroids is that they circulate in at least two physical forms: as free steroids dissolved in the aqueous phase of plasma, and as complexes adsorbed to serum proteins. Most natural corticosteroids in fact do so in the bound form. Corticosteroid binding globulin (CBG) is a highly specific binder for this class of steroids, and the naturally occurring members of the class, and some synthetic analogs bind to CBG with high affinity (1). But corticosteroids also bind, with less specificity and affinity, to a number of plasma proteins. Because of its high plasma concentration, albumin represents a major adsorbent for circulating steroids. Thus about 10% of circulating corticosteroids are free and about 90% are bound. Evidence in tissue culture systems strongly sug-

gests that it is the free steroid which acts upon target cells (2-5). This evidence includes the following points, obtained from our studies in a cultured line of rat hepatoma cells (HTC cells) which express the classic glucocorticoid-induced enzyme tyrosine aminotransferase. This enzyme can be induced in these cells either in serum-containing medium or in defined medium from which all proteins have been removed (2, 3). In fact, if limiting quantities of the inducing steroid are added to the cells, CBG and CBG-containing sera can be shown to reduce the extent of enzyme induction, presumably by binding a portion of the steroid in a form not available to the cells (4). Furthermore, the added potency of certain synthetic steroids can be partially explained by their lack of binding to CBG as a result of which, a higher concentration of free steroid reaches the target cell. On the other hand, the large pool of circulating, bound, native steroids may provide a relatively stable supply of hormone, damping out large and rapid variations in free steroid concentration and thus ensuring a modulated tissue response (1, 6). The obvious consequence of these considerations is that any substance altering the dynamics of the cardiovascular system or the synthesis and turnover of the serum proteins may alter the tissue content of free steroid and therefore steroid-mediated cellular activities. Such alterations would be predicted to be most marked following acute changes in the above parameters, since chronic alterations in free steroid concentration would eventually be compensated for by adjustments in synthesis and excretion.

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Cell Uptake and Metabolism of Corticosteroids

Most experts in the field believe that cell uptake of steroids is a passive process (1). Free steroid in the tissue space is thought to dissolve in the plasma membrane and then to reappear in the cytoplasm, due to its tight binding to a specific receptor (of which, more below). Harrison, however, has presented data in one cell system at least which suggests that uptake is an active, energy-requiring process (1). In my opinion, the question of cell uptake of steroids is underinvestigated. In any case, alterations of target cell membranes by toxic chemicals could produce changes affecting steroid uptake.

Metabolism of glucocorticoids is rarely if ever required to produce an active intracellular compound. Much data indicate that in most cells active glucocorticoids produce their responses without alterations in other structures. However, corticosteroid metabolism to less active forms may play a critical role in determining the tissue effects of the hormone. A clear example of this is found in the work of Santen et al. (7, 8). These workers were interested in testing the effects of aminoglutethimide to bring about a "medical adrenalectomy" for the treatment of breast cancer. The goal was to eliminate adrenal-derived estrogens, and of course it was necessary to provide replacement glucocorticoids for the patients, since the blocker inhibits all

steroid synthesis. Initially dexamethasone was chosen as the replacement. This is a highly potent glucocorticoid, not normally metabolized to less active form(s). But in the aminoglutethimide-treated individuals, it was found that dexamethasone was metabolized/excreted and was therefore ineffective in replacement therapy. In fact the natural glucocorticoid, cortisol, actually provided better replacement therapy. The conclusion which must be drawn from this example is that not only may normal metabolism and/or excretion of corticosteroids be altered by unusual compounds, but also that unexpected metabolic paths may be induced, grossly changing the actions of the natural or synthetic adrenal steroids.

Intracellular Actions of Corticosteroids

The initial intracellular interaction between steroid and cell elements is that of binding to receptor. These receptors are proteins specific for each class of steroid. Thus the receptors for corticosteroids are proteins of 80-100,000 daltons, highly sensitive to thiol oxidants, and having one high-affinity glucocorticoid binding site per molecule (9). In most systems studied, the affinity for potent glucocorticoids (expressed as apparent equilibrium dissociation constant) is $K_d \sim 10^{-9}$ - $10^{-8}M$. Occupancy of these proteins by steroid correlates well with

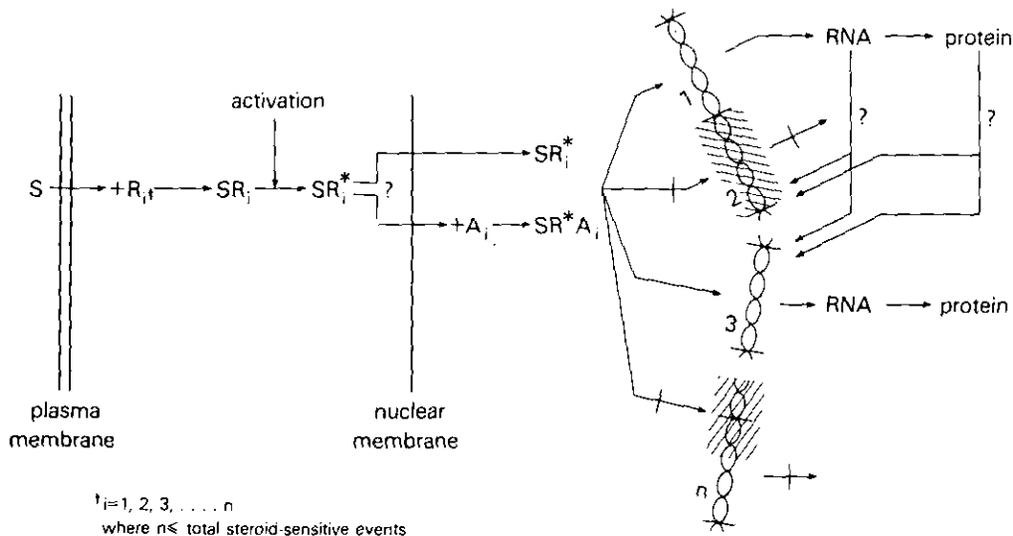


FIGURE 1. Generalized model for steroid hormone action. Steroid freely enters cell, where it combines with free receptor, R, to make initial steroid-receptor complex, SR. The complex undergoes activation to the SR* form, which binds in the nucleus due to increased affinity for DNA/chromatin. The nuclear complex, NR results in alterations in RNA synthesis and therefore in altered cellular enzymes, etc. This simple model does not explain differentiated cellular responses to a specific steroid but some possibilities are pointed out in Figure 2.

extent of specific cell response, and steroid-receptor affinity predicts well the relative potencies of most steroids. Historically, the receptor concept as applied to steroids developed from observing concentration of radiolabeled estrogens in known estrogen target tissues (uterus, oviduct, etc.). Subsequently, receptor proteins were indeed found in relatively high concentrations in those tissues. The idea was advanced that the simple presence or absence of receptor in a cell or tissue was critical in determining response to a sex steroid (10). This view has gradually changed subsequently. Certainly it is clear that virtually all normal tissues contain glucocorticoid receptors (and probably sex steroid receptors too), although their concentration varies between cells. The currently unanswered question of mechanism is, therefore, what lies beyond the receptor. Putting it another way, what mechanisms account for the diverse tissue responses to corticosteroids, considering the fact that all or most cells contain receptors for these steroids? Figures 1 and 2 show a model of steroid action which attempts to point out some of the possible sites for this diversity of response.

Tissue culture systems have been particularly useful in defining certain aspects of the actions of glucocorticoids. The idea that such steroids act by inducing enzymes indeed came from *in vivo* studies (11, 12), but in HTC cells it was possible to show that the hormones acted directly on the target cell (2, 13). Subsequently an estimate of the limits of the effects of glucocorticoids on cell proteins was obtained by displaying HTC cell proteins by the technique of two-dimensional gel isoelectric focusing/electrophoresis. Comparison of steroid-treated and untreated samples showed that some proteins appeared to be induced while others were reduced after steroid. The total number of altered peptide spots was of the order of 20 (14). Isolation of cell variants from the HTC parent line showed that different steroid responses could be under separate controls. That is, cells selected because of their lack of one response did not necessarily lack all other responses to glucocorticoids (15).

One of the classical cell responses to corticosteroids is lysis of thymus-derived lymphocytes (16). This property has been exploited in tissue culture systems to select steroid-resistant cells. Nearly all the cells so isolated have shown an abnormality of their steroid receptors (17-19). The usual phenotype in steroid-resistant mouse lymphoid and human leukemic cell lines has been loss or functional abnormality of receptors for the steroid. Of particular interest has been the finding that some human leukemic cells with receptors still able to bind steroid are nevertheless steroid-resistant. When

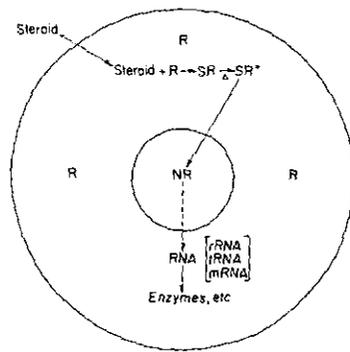


FIGURE 2. Possible mechanisms of noncoordinate control of multiple steroid-sensitive functions. This model assumes that (1) all the functions investigated are effected through steroid-receptor-chromatin interactions; (2) steroid enters the cell by free diffusion; (3) there is no selective action at the nuclear membrane; and (4) the affinity of receptor for steroid is identical, whether a single or many classes of receptor exist. These assumptions may be false, but are made here to focus attention on steroid-receptor interactions in the cell. After entering the cell, steroid (S) binds to receptors (R). At this level and beyond, several nonexclusive possibilities may intervene: (1) There are two or more subsets of receptors for the steroid ($R_1, R_2, R_3, \dots, R_n$). These subsets vary only in the facet of their surface which interacts with other cellular sites. With respect to steroid affinity, molecular weight, pI, etc., they closely resemble one another. The variation in the nonsteroid binding surface between receptor subsets accounts for their interaction with various chromatin sites, resulting in specific cellular responses. Thus, SR_1 might be responsible for TAT induction and PDE suppression, SR_2 for GS induction, SR_3 for AIB inhibition, etc. (2) There exist, as has been suggested in several steroid-sensitive systems, intermediate proteins (A_i) between the steroid-receptor complex and the DNA. These intermediaries could fulfill the role of providing multiple specificities by providing a variety of sites capable of recognizing distinct DNA sites. (3) There could be preferential masking of regulatory sites on the DNA (curved lines). Thus, if 1, 2, 3, \dots , n are n genes controlled by glucocorticoids, the shading on 2 and on the regulatory end of n represents masking proteins preventing SR^* ($\pm A$) interaction with the genome. Finally, (4) there may be regulatory loops, with control, either positive or negative, exerted by the products of certain induced genes. This is indicated by the arrows from RNA and protein on the right, directed back towards the DNA. Taken in part from Thompson, et al. (27).

examined in broken-cell systems, however, the receptors from these cells were found to be abnormally labile (20). Cells such as these may account for at least some classes of human receptor-positive, steroid-resistant leukemia.

The fact that most cells contain corticosteroid receptors and yet show a wide variety of specific responses to the hormones at once announce the likelihood of post-receptor controls. These may be positive-acting, allowing or causing the steroid-receptor complex to exert a particular intracellular action, or negative, blocking a response or set of

Table 1. Glucocorticoid-inducible peptides whose expression has been suppressed in somatic cell hybrids.^a

Peptide	Cross(es)
Tyrosine aminotransferase	Rat × rat, mouse, human, Chinese hamster
Tryptophan oxygenase	Mouse × mouse
Alanine aminotransferase	Rat × Chinese hamster
Growth hormone	Rat × mouse
Glycerol-3-phosphate dehydrogenase	Rat × mouse

^a Detailed references available elsewhere (21).

responses. Evidence for the latter kind of controls comes from somatic cell hybrids. In a number of crosses between cells responding to corticoids by induction of a particular peptide with cells not so responding, induction of the peptide has been lost (Table 1). In the case of estrogen induction of prolactin the same is true, and there we have shown that the hybrid cells contain no translatable prolactin mRNA (22). Thus, the control exerted probably prevents the cell from accumulating the relevant mRNA. Agents under test for toxicity might alter cellular responses to steroids by causing altered receptor content or function or by causing adaptive or mutational changes in the receptor gene. They might also cause changes in cellular regulatory machinery, producing abnormal responses or lack of response to an otherwise normal steroid-receptor unit.

Intercellular and Multihormonal Effects

The interwoven intercellular interactions and multiple layers of hormonal controls which are currently the focus of great attention in endocrinology and cell biology behoove the toxicologist to be aware of possible secondary and tertiary effects of the agent he studies. For example, as stated above, thymic lymphocytes are inhibited and even killed by pharmacologic concentrations of corticosteroids. Subsets of such "T-cells" function in the immune system as "killer lymphocytes," or as helpers or suppressors of B cells, the lymphocytes which synthesize circulating immunoglobulins. Furthermore, the growth and proliferation of T-cells depend on the production of thymic cell growth factor by other cells of the thymus (23). The synthesis of this factor is inhibited by corticosteroids (24). Thus the apparent effects of the corticosteroids on the immune system could be radically altered by compounds which themselves vary any of several cell or hormonal elements within that system. The inter-

dependence of corticosteroids and other hormones has been well known for years (5, 25). One example from recent research will serve to illustrate this interdependence. GH cells are a tissue culture line of rat pituitary adenoma cells. In these cells, corticosteroids alone have only a slight inductive effect on the production of growth hormone. When thyroid hormone and corticosteroids are given together, however, there is a marked synergism in the production of growth hormone (26). The action of the adrenal steroid is strongly modified by the presence of the thyroid hormone. Diminished effect of the steroids in certain systems following exposure to toxic products could well come from secondary hormonal interactions.

Summary

In this article, I have briefly set forth some of the factors upon which the function of adrenocortical hormones depends. These include delivery by the circulatory system, binding proteins in the circulation, metabolism of the hormones, intracellular receptors and modifiers of cell response to the steroid-receptor complex, cell-cell interactions, and multihormonal interactions at the target cell. Consideration of all these factors is necessary in appraising the effect of potentially toxic materials on corticosteroid function.

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