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PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF STEROIDS ON THE FUNCTION OF THE ADRENAL CORTEX

PETER J. HORNSBY

Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912, U.S.A.

Summary—The adrenal cortex is the site of the synthesis of steroid hormones such as the glucocorticoid cortisol and the mineralocorticoid aldosterone. The pathway of biosynthesis of these steroids from cholesterol involves a sequence of transformations using cytochrome P-450 enzymes which varies within the adrenal cortex as a result of the differential localization of enzymes within the zones.

The hypothesis presented here is that as a result of the arrangement of the vasculature in the adrenal gland, high concentrations of steroids may be expected to accumulate and may have autoregulating effects. These may include the following:

(1) the normal morphological and functional zonation of the adrenal cortex may be regulated by gradients of steroids in the adrenal cortex;

(2) destruction of cytochrome P-450 enzymes on interaction with certain steroids which act as pseudosubstrates may form part of the pathogenesis of some steroidogenic enzyme deficiencies. Under normal conditions, the individual cytochrome P-450s are not rate-limiting for steroidogenesis. Under some pathological conditions, individual cytochrome P-450 enzyme activities may become rate-limiting, with consequent overproduction of precursor steroids, leading to mineralocorticoid or androgen excess.

REGULATION OF STEROIDOGENESIS BY STRUCTURE

The regulation of the structure of the mammalian adrenal cortex is an intrinsic component of the regulation of the synthesis of the adrenocortical steroid hormones. Adrenocortical structure is involved in the regulation of the synthesis of the mineralocorticoid, glucocorticoid and androgenic steroids secreted by the adrenal, under both normal and pathological conditions.

The rate of secretion of any particular adrenocortical steroid is determined by the product of its rate of synthesis, per unit volume of adrenocorticol tissue, and the volume of adrenocortical tissue involved in its synthesis; this may not comprise the entire cortex but may consist of one or more zone or parts of zones. In turn, the rate of production of a given adrenocortical steroid is determined by the product of

(1) the total rate of synthesis of all steroids in the part of the cortex involved in its synthesis and
(2) the proportion of total steroid output that the steroid represents within this part.

The rate of total steroidogenesis is determined by the rate of supply of cholesterol to the cytochrome P-450 that cleaves its side-chain to yield pregnenolone. The rate of flux through the rate-limiting step determines the rate of synthesis of the sum of the steroid products, but does not determine the rate of synthesis of any individual steroid.

The relative activities of the enzymes of the steroidogenic pathway beyond the formation of pregnenolone determine the pattern of steroidogenesis, i.e. they determine which steroids are produced and in what ratio.

A mechanism for regulation of steroidogenesis is the volume of the zones

We have proposed that a major mechanism for the long-term regulation of adrenocortical steroidogenesis is the regulation of the volume of the various zones [1]. This level of regulation of steroidogenesis has frequently been overlooked. The volume of the zona glomerulosa is a major factor for the long-term regulation of aldosterone synthesis, and the volume of the inner zone (zona reticularis or fetal zone) is of major importance for the regulation of adrenal androgen synthesis. To some extent the volume of the zona fasciculata is also of importance for the regulation of glucocorticoid synthesis, but here the volume of the cortex as a whole is the important morphological factor.

Width is regulated by gradient, volume indirectly by feedback

Whereas it is zonal volume which is the determining morphological factor in control of steroidogenesis, we propose that volume as such is not the parameter that is under primary regulation. Rather, zonal width is likely to be the primary regulated factor, if the gradient model of zonation is correct, as discussed below. Zonal volume would be regulated indirectly by the combination of (1) the regulation of the growth and size of the adrenal cortex as a whole and (2) by regulation of zonal width by regulation of the rate of synthesis of the gradient substance(s). An individual hormone, e.g. ACTH, could be involved in both of these processes.

THE VASCULAR SYSTEM AND THE GRADIENT HYPOTHESIS

Zones result from an environmental influence

It was first proposed by Greep and Deane in 1949 that gradients created by the adrenal cortex's unusual pattern of capillaries may be involved in zonation, and that the division of the cortex into the mineralocorticoid- and glucocorticoid-secreting zones may be the result of a requirement for different local cellular environments for the synthesis of the different steroid hormones, as provided by the centripetal capillary system [2]. Discussing regeneration of the zones from the zona glomerulosa after adrenal enucleation, they write: "These observations... raise again the basis for that zonation. Apparently, cortical cells originating from the peripheral region, whether in normal organogenesis or after enucleation of the gland, have different functions at various levels from the surface. Is the formation of 11-oxygenated hormones [glucocorticoids] dependent upon the presence of desoxycorticosterones [mineralocorticoids] in the blood bathing the cells? Does the formation of the desoxycorticosterone-like hormones require more oxygen, more foodstuffs and fewer waste products than the formation of 11-oxygenated hormones? Or... does the age of the cell in some way influence the nature of its product?" Although differing in detail, current concepts of the origination of functional zonation are very close to these original ideas.

A gradient across the capillary bed

We and others have hypothesized that adrenocortical cells produce a gradient of a substance or substances in the bloodstream which alter adrenocortical cell function and morphology to create the zonation of the adrenal cortex [1, 3–15]. The concept is illustrated in Fig. 1. This hypothesis is presented as an attempt to integrate a wide range of adrenocortical cell biology.

Steroids reach high concentrations within the adrenal cortex

Adrenal arterial plasma steroid concentrations are, of course, those of the general peripheral circulation, whereas very high concentrations of steroids are found in the adrenal venous effluent. Glucocorticoid concentrations in adrenal venous plasma are of the order of 60 μM [14].

Steroids may form a gradient

The substances forming a gradient across the capillary bed are postulated to be steroids secreted by the adrenocortical cell. It is simpler to construct a model for zonation if the gradient substance is assumed to be a steroid, particularly a glucocorticoid. The postulate of steroids as the constituent of the gradient is a specific concept within the general hypothesis that zonation results from the action of a substance secreted by adrenocortical cells creating a gradient across the capillary bed. However, several aspects of the hypothesis do depend on the gradient substances being responsive to stimulation by ACTH.

THE ZONA GLOMERULOSA

Part of the physiological regulation of the synthesis of the mineralocorticoid aldosterone is the regulation of the volume of that part of the adrenal cortex involved in its synthesis. This volume, to a good approximation, is the volume of the zona glomerulosa, since there is reasonable evidence that the synthesis of this steroid is confined to this zone.

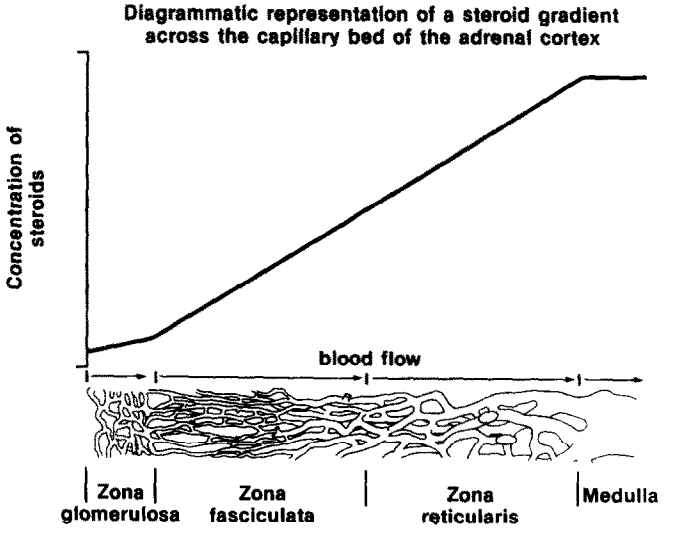

Fig. 1. Diagrammatic representation of a steroid gradient across the capillary bed of the adrenal cortex.

The zona glomerulosa is the site of aldosterone synthesis

It was realized in the 1940s that the width of the zona glomerulosa varied with the state of the salt and water balance of the animal and it was concluded that this zone of the cortex was responsible for the secretion of a hormone involved in regulation of these functions [16]. Deoxycorticosterone (DOC) had been isolated and was believed to be the salt-retaining steroid hormone. Only in the 1950s was the physiological salt-retaining hormone, aldosterone, isolated and characterized [16], and *in vitro* experiments conclusively established that the mechanically separated zona glomerulosa was the only site of synthesis of this steroid in the cortex [17]. So far as can be established using microdissection techniques, there is a correspondence between the zona glomerulosa and the aldosterone-secreting zone [11, 17–19].

Aldosterone secretion results from the presence of a cytochrome P-450 species with corticosterone methyl oxidase (CMO) activity. It has been thought that CMO results from the activity of a separate cytochrome P-450 (P-450_{CMO}); but there is evidence that CMO is actually an activity of cytochrome P-$450_{11\beta}$ [20, 21]. The presence of a particular lipid or other cofactor may be required to confer CMO activity on cytochrome P-$450_{11\beta}$. In the absence of this factor, cytochrome P-$450_{11\beta}$ synthesizes corticosterone, 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone from deoxycorticosterone, but not aldosterone [22, 23]. Here, the term "CMO activity" will be used to indicate the activity of cytochrome P-$450_{11\beta}$ acting together with whatever accessory factors are required for aldosterone synthesis. Regulation of CMO activity differs from regulation of 11β-hydroxylase activity. CMO activity is increased on exposure of adrenocortical zona glomerulosa cells to elevated medium [K^+] whereas 11β-hydroxylase activity, but not CMO, is increased after incubation with ACTH or other agents which raise intracellular cyclic AMP [3, 4, 24].

The other significant difference in cytochrome P-450 content between the zones is the absence of 17α-hydroxylase in the glomerulosa and its presence in the fasciculata, except in those species (principally the rat and some other rodents) that lack 17α-hydroxylase throughout the adrenal cortex [18, 19, 25, 26]. $C_{17,20}$-Lyase is also present only in the inner zones; this enzymatic activity is probably catalyzed by the same cytochrome P-450 [27, 28].

Zona glomerulosa is always on the arterial side of the capillary bed

The zona glomerulosa is always present only on the extreme arterial side of the capillary bed. This is true in several unusual situations other than the normal gland.

(1) In the human adrenal gland, a cuff of cortex surrounding the central vein penetrates the medulla. The blood supply in the cuff is from a plexus that surrounds and supplies the wall of the central vein. Capillaries radiate outwards from this plexus supplying the cortical cuff. A zona glomerulosa occurs on the arterial side of the capillary bed [14].

(2) Accessory adrenal glands are miniature glands consisting only of adrenal cortex, no medulla being present, and are frequently present as developmental abnormalities in some strains of animals [29]. They are normally small, but are capable of dramatic growth when the animal is adrenalectomized; when accessory glands are present, the usually lethal process of adrenalectomy is survived. When accessory adrenal glands are large enough to show an internal structure, the capillary bed is seen to have its normal structure with the zona glomerulosa on the arterial side [30].

(3) Adrenocortical tissue that has regenerated from tissue fragments also shows a glomerulosa on the arterial side of the capillary bed [31]. This zone reforms after most of the regenerative growth is completed. During regenerative growth, structure is disorganized, and zonation of the adrenocortical tissue is not apparent.

(4) When adrenocortical tissue is transplanted into the anterior eye chamber, the grafts may revascularize from the iris and thus receive their blood on one surface of the graft only. A zona glomerulosa is seen to form along this surface [32].

In these unusual circumstances functional, as opposed to morphological zonation, has not been investigated, although in certain cases it may be inferred that aldosterone secretion is intact.

The glomerulosa–fasciculata boundary determined by the gradient

If zonation of CMO activity and other glomerulosa-specific functions is the result of a gradient of steroids across the adrenocortical capillary bed, then presumably the gradient steroid reaches a critical concentration at the zona glomerulosa/zona fasciculata boundary, supplying a signal to suppress the expression of glomerulosa-specific functions in the zona fasciculata.

It has long been observed that the relative width of the zona glomerulosa and the zona fasciculata is under regulation by ACTH. Administration of ACTH, while causing hypertrophy of the entire cortex, causes a diminution in the width of the zona glomerulosa [29]. This observation led to the "transformation field" hypothesis in which the borders of the glomerulosa and fasciculata (and also fasciculata and reticularis) were hypothesized to be changeable, with the width of the fasciculata increasing at the expense of the other zones to supply an increased demand for adrenocortical steroids [29]. This is similar to the concept, still sometimes encountered, that the zona glomerulosa

is an "undifferentiated" reserve zone for the "differentiated" fasciculata. This concept is untenable in the light of the highly specialized functions of the zona glomerulosa and its unique enzyme activities. Nevertheless, it does appear that the movement of the border between the glomerulosa and the fasciculata under the action of ACTH is an important regulatory mechanism for homeostasis of mineralocorticoid output.

When the width of the adrenal cortex as a whole increases during long-term growth under the action of ACTH, the width of the glomerulosa must decrease in order to maintain a constant volume of the zone. ACTH stimulates the production of the gradient steroid (e.g. corticosterone), raising the concentration of the steroid in the gland and causing a change in the shape of the gradient across the capillary bed. The distance from the capsule at which the concentration of steroid becomes sufficient to signal suppression of glomerulosa-specific functions will decrease, and thereby the width of the glomerulosa will decrease.

Regulation of the glomerulosa–fasciculata boundary by sodium depletion and angiotensin

It is somewhat difficult to distinguish an increase in the mass of the glomerulosa, due to a specific mitogenic action of angiotensin on this zone, from a movement inward of the boundary between the glomerulosa and the fasciculata or an increase in cell size in the glomerulosa [1]. Chronic administration of angiotensin causes an increase in adrenocortical mass [33], and sodium deprivation, known to cause an increased secretion of angiotensin, results in an increase in the adrenocortical mitotic index [34]. Whereas both inner and outer zones of the adrenal cortex have angiotensin receptors and show steroidogenic and mitogenic effects [35–37], there are more angiotensin receptors in the zona glomerulosa [38], and, at least in some systems, the steroidogenic effect of angiotensin is greater in this zone [35, 39]. This zonation of angiotensin receptors might be expected to result in a greater mitogenic effect on the glomerulosa than on the zona fasciculata. An increase in mitoses in this zone does indeed occur during sodium depletion [34]. However, mitoses in the adrenal cortex occur mainly in a region of the outer adrenal cortex, including the zona glomerulosa but not confined to it, and ACTH administration increases mitoses in this region [40, 41]. Sodium depletion also resulted in an increase in the mitotic index of the zona fasciculata [34]. *In vivo* there may be complex interactions between angiotensin and ACTH, leading to over- or underestimation of the direct mitogenic effect of angiotensin [1]. Thus, an effect of angiotensin on mitoses in the glomerulosa is not necessarily either specific or a direct action of angiotensin.

We have pointed out that there are several mechanisms by which an increase in the stimulation of the glomerulosa by angiotensin and $[K^+]$ may lead to a local decrease in glucocorticoid levels in this zone [1]. Thus, it is possible that changes in the boundary between the zona glomerulosa and zona fasciculata under changes in plasma ion levels and circulating angiotensin may be regulated by changes in the steroid gradient across the cortex, rather than specific mitogenic effects on glomerulosa cells.

Glomerulosa–fasciculata interconversion in culture

Culture experiments support the concept of the conversion of cells from a glomerulosa phenotype to a fasciculata phenotype. We have isolated zona glomerulosa cells and placed them in culture [3, 4, 10, 11, 42]. Treatment with ACTH causes loss of aldosterone synthesis (CMO activity), while causing large increases in 17α-hydroxylase activity [3, 4, 10, 11, 26, 42].

Aldosterone-secreting tumors and nodules

An extension of this concept to account for the mixed steroidogenic pattern of tumors secreting aldosterone has been discussed elsewhere [14, 43]. Briefly, disorganized growth of the cortex may give rise to nodules or benign tumors. When such a tumor has a blood supply relatively close to the arterial side of the capillary bed, it has the potential for secretion of aldosterone in those parts of the tumor. Other regions of the tumor that are not close to the arterial side of the bed will act as fasciculata cells and secrete cortisol. If no part of the tumor is close to the arterial supply, only cortisol is produced. This may result in a "non-functioning" nodule; such nodules are in fact functional, but the amount of cortisol they secrete is insufficient to alter the glucocorticoid balance of the body as a whole. On the other hand, aldosterone secretion by such tumors is likely to cause symptoms of aldosterone excess, because of the normally very low levels of secretion of this steroid.

Gomez-Sanchez [44] has made the interesting suggestion that in glucocorticoid-suppressible aldosteronism there is a failure of the normal suppression of CMO activity by steroids in the outer fasciculata, resulting in a zone with both CMO and 17α-hydroxylase activities. This would account for the synthesis of 18-hydroxycortisol and 18-oxocortisol in this syndrome [44, 45].

THE ZONA RETICULARIS

For those species in which the adrenal cortex secretes large quantities of androgenic steroids, there is evidence that most of the biosynthesis of these steroids is localized to the zona reticularis or other inner zones, and thus to some extent the volume of these zones is a regulatory factor for adrenal androgen production. However, since adrenal androgens may also be synthesized by the zona fasciculata, regulation of adrenal androgen synthesis by the volume of the zona reticularis is not as

unequivocal as regulation of aldosterone production by the volume of the zona glomerulosa.

Is there a coincidence of morphological and functional zonation?

Although the association between the zona reticularis and adrenal androgen synthesis has been popular for some time, it is not clear that there is a simple correspondence between the region of the adrenal cortex that secretes adrenal androgens and the morphological zona reticularis. A major obstacle to such a simple association is that a zona reticularis occurs in species that do not secrete appreciable quantities of adrenal androgens, such as the rat, cow, and many other species. The different enzyme levels in the inner zones result in a greater rate of adrenal and many other species. In androgen-secreting species, the different enzyme levels in the inner zones result in a greater rate of adrenal androgen synthesis relative to that of the zona fasciculata, or to that of the definitive zone in the fetal cortex. This has been shown directly by separate culture of the fasciculata and the reticularis, and of the definitive and fetal zones [46–48]. Cells from the zona reticularis or the fetal zone in culture initially produced greater quantities of dehydroepiandrosterone sulfate (DHEAS), and Δ^5,3β-hydroxy and sulfated steroids generally, than the zona fasciculata or the definitive zone. Zona fasciculata cells do secrete adrenal androgens but not at the same rate as reticularis cells [46].

Zonation of 3β-hydroxysteroid dehydrogenase (3β-HSD), sulfotransferase, and 17α-hydroxylase

It is clear that in the human, other primates, and some other species there is an association between the development of large glands with distinct inner zones (the fetal zone in fetal life, and the zona reticularis in maturity) and adrenal androgen synthesis [14, 49]. The important enzymatic features of functional zonation in the inner zones in the human adrenal cortex are low 3β-HSD, and high DHEA sulfotransferase, 17α-hydroxylase and $C_{17,20}$-lyase activities [9, 50–57]. In the guinea pig there also appears to be zonation of $C_{17,20}$-lyase and DHEA sulfotransferase [19, 57–59]. Synthesis of androgens in the human adrenal cortex results from primarily from the ratio of these critical enzymes [60].

Association of inner zones with androgen synthesis in the fetus and in adrenarche

In humans, the synthesis of adrenal androgens is associated with the development of prominent inner zones in fetal life and in maturity but not in childhood. Between birth and adrenarche DHEAS levels are very low, coinciding with the lesser development of the zona reticularis in this period [14]. 17α-Hydroxylase and $C_{17,20}$-lyase activities are also low before adrenarche, and then increase [9, 61–63].

The steroid gradient and the fasciculata–reticularis boundary

The mechanism by which a steroid gradient might result in a fasciculata–reticularis transition is speculative [1, 9, 15]. As for the glomerulosa–fasciculata boundary, there may be a critical point in the steroid gradient that results in transition from fasciculata to reticularis. Presumably, the adrenocortical cell has a mechanism for sensing the increased steroid concentration, resulting in changes in enzyme levels characteristic of the zona reticularis. Adrenarche, as has been previously suggested [9], may be the result of the increasing size of the adrenal cortex during childhood, perhaps simply reflecting a higher chronic level of stimulation with ACTH, with eventual achievement of a critical size where the concentration of the gradient substance in the inner cortex is sufficient to cause the development of the reticularis. This development could become self-sustaining in the following way. ACTH may stimulate an increase in adrenocortical size; stimulate an increase in the highly ACTH-dependent 17α-hydroxylase and the $C_{17,20}$-lyase activities; and cause a decrease in the 3β-HSD level in the inner cortex via the sensing of the gradient steroids. Thus ACTH will stimulate mostly DHEA(S) and less glucocorticoids by the reticularis. The lack of glucocorticoid response will tend to increase ACTH secretion by the pituitary, and so complete a positive feedback cycle. Consequently, it may be proposed that quite small initial changes in ACTH secretion may result in large changes in the reticularis and adrenal androgen synthesis.

Summary

Thus, there appears to be a single parenchymal cell type in the adrenal cortex, exhibiting phenotypic modulation according to the position of the cell within the adrenal vascular system.

BIOLOGY OF PSEUDOSUBSTRATE EFFECTS IN ADRENOCORTICAL CELLS IN CULTURE

If a steroid gradient is involved in regulation of adrenocortical zonation, adrenocortical cells must be able to sense their position in the steroid gradient, and thus their position within the cortex. Two mechanisms for this sensing are possible: there may be steroid receptors, analogous to receptors in peripheral tissues, but clearly having the property of requiring much higher concentrations for saturation and activation; alternatively, adrenocortical cells may use cytochrome P-450–pseudosubstrate interactions to regulate zonation.

Several of the cytochrome P-450 enzymes involved in steroidogenesis in the adrenal cortex are destroyed on interaction with pseudosubstrate steroids. Pseudosubstrates are compounds which bound by cytochrome P-450 enzymes like substrates

but which cannot be hydroxylated because of steric or chemical reasons [15, 64, 65]. We have studied pseudosubstrate effects in several cytochrome P-450 enzymes (cytochrome P-$450_{11\beta}$, cytochrome P-450_{C21}, and cytochrome P-$450_{17\alpha}$) in cultured bovine and human adrenocortical cells. From these studies and work in other laboratories, the following generalizations may be made about pseudosubstrate effects in cytochrome P-450 enzymes, which distinguish pseudosubstrate effects from other modes of regulation of these enzymes.

Characteristic time-course of loss of activity

Loss of activity begins immediately on adding the pseudosubstrate steroid to the cells, but is not usually complete before ~24 h [8, 11, 13, 66, 67] (Fig. 2).

Pseudosubstrates do not act via receptors

Several lines of evidence indicate that the action of pseudosubstrates is one of a direct action on the cytochrome P-450 enzyme, and is not via the action of a protein receptor which mediates changes in gene activity. Pseudosubstrate effects do not require protein synthesis [67]; pseudosubstrate structure is not related to receptor binding affinity, but rather indicates that pseudosubstrates interact with the enzyme substrate site [8, 13, 67]; and the concentrations of pseudosubstrates required are much greater than would be expected for saturation of the known forms of receptors [8, 13] (Fig. 3).

Pseudosubstrate effects do not result from inhibition of the enzyme

Loss of activity does not result from reversible inhibition of the enzyme. Pseudosubstrates are poor inhibitors of enzyme activity when added to cultures together with substrate [8, 13, 67].

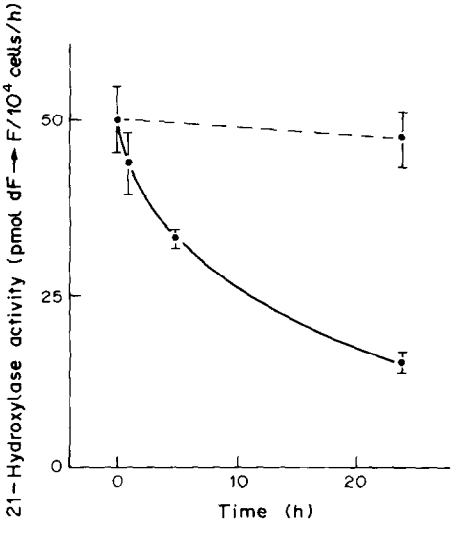

Fig. 2. Time course of loss of 21-hydroxylase activity.

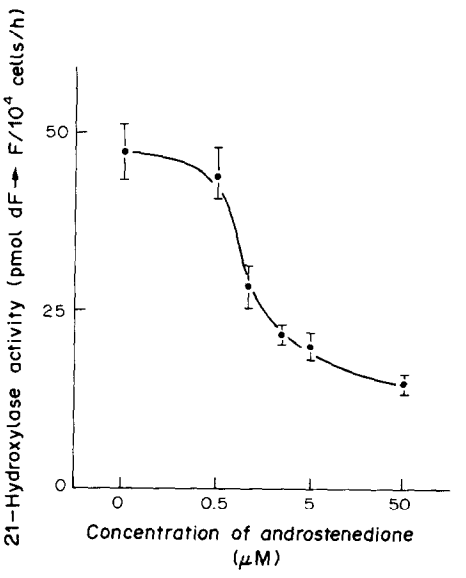

Fig. 3. Effect of substrate concentration (androstenedione) on 21-hydroxylase activity.

Enzyme activity does not recover after removal of pseudosubstrate

No recovery of enzyme activity is seen when the pseudosubstrate is removed from cultures after exposure; but enzyme activity may be restimulated on exposure to a suitable inducer such as ACTH [67].

Inhibitors and antioxidants protect enzyme against the pseudosubstrate effect

Inhibitors of the cytochrome P-450 enzymes generally protect against loss of activity in the presence of pseudosubstrates [10, 11, 13, 68–70]. Presumably one or more of the following mechanisms are involved: preventing the pseudosubstrate from binding at the substrate site; preventing the formation of destructive oxidants [15, 64, 65]; or inhibiting the peroxidase activity of the enzyme (see below).

Degradation of the protein moiety of the cytochrome P-450

Although the actual change in the cytochrome P-450 enzyme which results in loss of activity is not known, the protein moiety of the enzyme is observed to be degraded, when examined by Western blotting [71], perhaps as a result of the action of proteases which recognize damaged proteins [72].

Damage by peroxidation

If superoxide is formed by the cytochrome P-450 species when attempted metabolism of pseudosubstrates occurs and normal hydroxylation cannot take place [64, 65, 73], superoxide or its products could damage the enzyme. If superoxide initiates lipid peroxidation, damage to cytochrome P-450 enzymes may result from peroxidase activity of cytochrome P-450 [64, 65, 73].

POSSIBLE PATHOLOGICAL EFFECTS OF PSEUDOSUBSTRATE EFFECTS

Regardless of the possible regulatory roles of pseudosubstrate interactions in zonation, pseudosubstrate effects may be involved in experimental and human syndromes of cytochrome P-450 enzyme deficiency, with resultant overproduction of deoxycorticosterone (DOC) or androgens.

Since steroids are often present in high concentrations in steroidogenic tissues, the possibility that loss of 21-hydroxylase or 11β-hydroxylase through interaction with steroid pseudosubstrates can occur *in vivo* should be considered. Because progesterone and 17α-hydroxyprogesterone are intermediates in steroidogenesis, it is difficult to assess intracellular concentrations of these steroids; however, for androstenedione it is possible to assess whether intra-adrenal concentrations would be high enough to have pseudosubstrate activity *in vivo*. The concentration of androstenedione in adrenal venous blood is 3–28 μg/dl [14]. Allowing for 50% dilution with blood to the medulla that has not passed through the cortical capillary bed [14], the concentration of androstenedione in the inner region of the cortex may be 0.2–2 μM. This is within the range of concentrations of androstenedione that cause loss of 21-hydroxylase activity in cultured bovine adrenocortical cells and human adrenocortical cells [13]. The concentration of cortisol required to produce loss of 11β-hydroxylase in culture experiments ($\sim$10 μM) is high relative to concentrations in peripheral blood but is well within the range of glucocorticoid concentrations found in adrenal venous blood ($\sim$60 μM) [14]. Thus, the potential exists for intraadrenal glucocorticoids to depress 11β-hydroxylase, as a physiological or pathological mechanism.

Pathological effects of 11β-hydroxylase–pseudosubstrate interactions: excess DOC

There are examples of experimental 11β-hydroxylase deficiency in which pseudosubstrate effects appear to be involved. In these cases, deficiency of 11β-hydroxylase leads to excess DOC production with consequent hypertension. It is possible that similar processes could be involved in certain forms of human hypertension in which the adrenal cortex may be involved.

Androgen effects

In 1953, Skelton reported that administration of an androgen, methylandrostenediol, produced hypertension in rats [74]. Androgen-induced hypertension was found to be dependent on the presence of the adrenal glands, at least for its initiation [75, 76]. The finding that methylandrostenediol and its product, methyltestosterone, blocked 11β-hydroxylase by competitive inhibition, indicated that the resultant overproduction of DOC was the cause of the hypertension [77]. However, it was also shown that 11β-hydroxymethyltestosterone, not a competitive inhibitor of 11β-hydroxylase but rather a product of this enzyme activity, also caused hypertension [78, 79]. Moreover, there was a substantial decline in adrenocortical mitochondrial cytochrome P-450 content in the androgen-treated animals, indicating an actual loss of cytochrome P-450$_{11\beta}$, rather than simple inhibition [78, 79]. Administration of a potent inhibitor, metyrapone, did not produce this loss of cytochrome P-450 in the mitochondria [78, 79].

Rather than inhibition of 11β-hydroxylase as the cause of androgen-induced hypertension, it appears that a pseudosubstrate effect is responsible. In cultured bovine adrenocortical cells, 11β-hydroxylated androgens, such as 11β-hydroxytestosterones and 11β-hydroxyandrostenedione, were shown to be pseudosubstrates for 11β-hydroxylase [8], as well as the non-11β-hydroxylated forms, such as testosterone, 17α-methyltestosterone, and androstenedione [8, 80]. The loss of adrenal weight seen in androgen-induced hypertension is perhaps the indirect result of the cytochrome P-450–pseudosubstrate interaction.

An involvement of androgens acting as pseudosubstrates in human syndromes of DOC overproduction is not clear. However, it has been proposed that in classical simple virilizing and nonclassical 21-hydroxylase deficiency, excess adrenocortical androstenedione production can lead secondarily to loss of 11β-hydroxylase and excess DOC production by a pseudosubstrate mechanism, particularly under continued ACTH stimulation [81]. A positive feedback cycle could be established as illustrated in Fig. 4. Intradrenal androstenedione is likely in the range of concentrations that depress 11β-hydroxylase in cell culture experiments (1–10 μM) [8, 14].

Glucocorticoid effects

An example of a pathological depression of 11β-hydroxylase, probably mediated by glucocorticoids acting as pseudosubstrates, is the defective 11β-hydroxylation in the adrenal glands of animals implanted with ACTH-producing tumors [82]. Under chronic, excessive ACTH stimulation, as can occur in animals with ACTH-secreting tumors, the amount of DOC secreted by the fasciculata can become high enough to satisfy the mineralocorticoid requirement of the animal [83, 84]. The glomerulosa may then become limited to foci under the capsule, no longer forming a continuous layer [14, 85]. This indicates that under the combined effect of the chronic overstimulation with ACTH and lack of glomerulosa stimulation, the critical point in the gradient has moved outward to reach the capsule itself.

Additionally, 11β-hydroxylase has been reported to be low in the normal zona reticularis [58, 86].

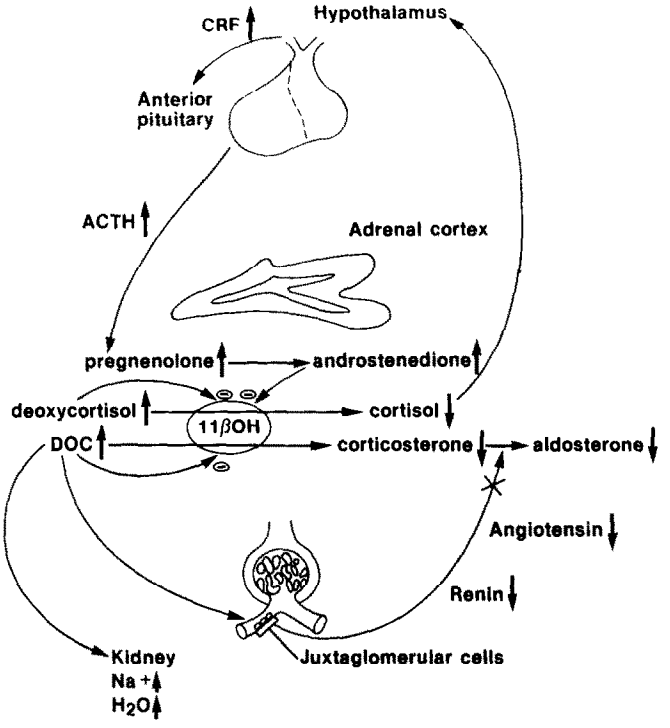

Fig. 4. Hypothesis for positive feedback cycle in DOC synthesis by adrenal cortex.

Pathological effects of 21-hydroxylase–pseudosubstrates

Since androstenedione does not suppress 17-hydroxylase or $C_{17,20}$-lyase activities [13, 67], the achievement of a sufficient concentration of androstenedione in the inner part of the cortex may suppress glucocorticoid formation while continuing to allow its own synthesis. A positive feedback cycle could be established whereby androstenedione suppresses 21-hydroxylase activity, causing decreased glucocorticoid production; lowered glucocorticoid production rates increase secretion of ACTH by the pituitary; ACTH then stimulates steroidogenesis, leading to more androstenedione secretion. This hypothesis is illustrated in Fig. 5.

Certain forms of 21-hydroxylase deficiency

A hypothesis for the behavior of 21-hydroxylase in some forms of 21-hydroxylase deficiency is that an altered primary sequence of the enzyme leads to an increased susceptibility to pseudosubstrate effects [13]. A change in primary sequence could lead to an enhanced susceptibility to loss of activity in the presence of pseudosubstrates in several ways; the following is not an exhaustive list. First, there could be decreased affinity for normal substrate, 17α-hydroxyprogesterone, with unchanged affinity for androstenedione, resulting in a greater ratio of non-productive to productive cycling. Second, there could be a change in the enzyme substrate site resulting in an enhanced tendency for normal substrate to act as a pseudosubstrate; for example, the substrate may bind such that the distance between substrate and heme oxygen is greater, leading to an impairment of oxygen transfer. Third, cytochrome P-450$_{C21}$ could have an enhanced tendency to suffer damage during non-productive cycling, such as a decreased ability to accept electrons from cytoch-

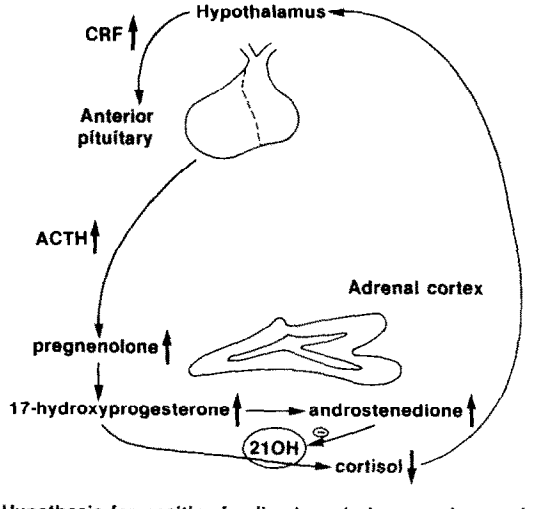

Fig. 5. Hypothesis for positive feedback cycle in some forms of 21-hydroxylase deficiency.

rome b_5, which has been postulated to discharge destructive oxygenating complexes in non-productive cycling [15, 64]. Fourth, an altered conformation of the enzyme might result in a change in the orientation of the protein in the membrane, with indirect effects on the ability of the enzyme to interact with pseudosubstrates or to be damaged by the interaction.

An altered susceptibility to pseudosubstrate effects would provide an explanation for the apparent distribution of the enzyme in the adrenal cortex in the simple virilizing and non-classical forms of 21-hydroxylase deficiency. In these forms, enzyme activity appears to be relatively intact in the zona glomerulosa but, to varying degrees, deficient in the zona fasciculata–reticularis. Because of the existence of gradients of steroids in the adrenal cortex *in vivo*, as discussed above, an altered 21-hydroxylase molecule might have an unusually short half-life due to the higher concentrations of pseudosubstrate steroids in the inner zones, such that to varying degrees there is deficiency of the enzyme in these zones, while having a normal half-life in the outer cortex, where pseudosubstrate steroid concentrations may be much lower or absent. This applies particularly to a pseudosubstrate synthesized by the 17α-hydroxylase pathway, which is absent in the zona glomerulosa [10, 26].

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