

Regulation of Aldosterone Biosynthesis: A Continual Challenge

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Aldosterone secretion by the zona glomerulosa of the adrenal cortex is directly influenced by a large, growing number of different physiologic agents. Their action includes: 1) acute stimulation or inhibition of early biosynthetic steps; 2) long-term activation or suppression of late biosynthetic steps; and 3) induction of growth or atrophy of zona glomerulosa cells. In rats, adaptation of late steps of aldosterone biosynthesis to sodium and potassium intake

is mediated by the induction or repression of a second form of cytochrome P-450_{11β}, which differs from the main form of the enzyme by a lower molecular weight and a greater range of catalytic properties. *Am J Hypertens* 1991;4:280–282

KEY WORDS: Sodium intake, potassium intake, rat, cytochrome P-450_{11β}.

A generation ago, the impact of the discovery of a close functional correlation between the renin–angiotensin system and aldosterone secretion was so extensive that angiotensin II is still widely considered to be the predominant if not the only physiologically important regulator of aldosterone production. However, already in 1960, the direct influences of ACTH and the extracellular potassium and sodium concentrations on the zona glomerulosa of the adrenal cortex had been clearly established. Today, at least 20 different substances of potential physiologic importance are known to directly stimulate aldosterone biosynthesis.¹ Their list includes peptides (angiotensin II and III, corticotropin, α -MSH (melanocyte-stimulating hormone), and other POMC derivatives, prolactin, vaso-

pressin, oxytocin, vasoactive intestinal peptide, neuro-peptide Y, endothelin), monovalent cations (K⁺, H⁺, NH₄⁺), amines (serotonin, histamine), acetylcholine, and prostaglandins. There is also a steadily growing number of substances reported to directly antagonize the action of aldosterone-stimulating agents. Among them, atrial natriuretic peptides are at present of particular interest, because of their high inhibitory activity demonstrated in the presence of all types of aldosterone stimulators and because of the known dependence of their secretion on the sodium and water balance of the mammalian organism. Perhaps, in the future, a ouabain-like natriuretic hormone might turn out to be an equally important physiologic aldosterone inhibitor.

MODE OF ACTION OF REGULATORS

The mechanism of action of the main aldosterone regulators on the zona glomerulosa cell has been extensively studied during the past 15 years and is still an open area of basic research.^{1–5} In principle, different stimulators interact with different receptors, which in turn activate different second messenger systems: adenylate cyclase, phospholipase C, or voltage-gated calcium channels. However, their final effect is the same, ie, the rapid and sustained facilitation of a biosynthetic step early in the pathway to aldosterone, preceding the formation of pregnenolone. In addition, most of the stimulators have

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Recent work from our laboratory has been supported by Research Grant 32.8572.85 of the Swiss National Foundation for Scientific Research.

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This article is based on data presented at the American Society of Hypertension annual meeting, May 18 to 21, 1990.

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been shown to have a minor short-term stimulatory effect on the conversion of corticosterone to aldosterone. Both of these effects can be blocked by atrial natriuretic peptides by a still unknown mechanism. These agents act through their own receptors and appear to block intracellular signal transmission at a site distal to the generation of second messengers.

LONG-TERM REGULATION OF LATE BIOSYNTHETIC STEPS

In man as well as in experimental animals, the response in aldosterone secretion to stimulators is variable and dependent on sodium and potassium intake. The activity of enzymes regulating late steps in aldosterone biosynthesis plays a main role in this long-term adaptation to alterations in sodium or potassium intake (see Figure 1). Thus, agents markedly stimulating aldosterone production in capsular adrenals of normally fed rats did not stimulate aldosterone production when added to capsular adrenals of potassium-deficient, sodium-loaded, or mineralocorticoid-treated rats, but elicited strikingly greater increments in deoxycorticosterone output.¹ This indicated that the activity of late steps in aldosterone biosynthesis involved in the conversion of deoxycorticosterone to aldosterone was rate-limiting and subject to changes in response to alterations in sodium and potassium intake. These changes mainly concerned the conversions of corticosterone to 18-hydroxycorticosterone and of 18-hydroxycorticosterone to aldosterone, ie, the so-called corticosterone methyl oxidations (CMO) 1 and 2, as well as the 11 β -hydroxylation of 18-hydroxy-11-deoxycorticosterone. The 11 β -hydroxylation or 18-hy-

droxylation of deoxycorticosterone were either not affected or affected only to a minor extent. These changes in late steps of aldosterone biosynthesis occurred slowly, in the course of days or at least several hours, and were characterized by a delayed onset. According to indirect evidence, the long-term regulation of late steps of aldosterone biosynthesis is also multifactorial. Whereas changes in potassium balance are mainly mediated by the extracellular potassium concentration, alterations in late steps in adaptation to the sodium status are partially mediated by the renin-angiotensin system, but additional unknown factors are also involved. Corticotropin, which is a potent stimulator of aldosterone biosynthesis in short-term experiments, has a long-term inhibitory effect on corticosterone methyl oxidations.⁶

A MITOCHONDRIAL PROTEIN DEPENDENT ON SODIUM AND POTASSIUM INTAKE

Resumption of potassium intake by potassium-depleted rats resulted in the appearance of a protein with a molecular weight of 49,000 in the mitochondria of the zona glomerulosa, but not in those of the inner zones of the adrenal cortex, as characterized by a distinct band revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.⁷ Over 48 h of potassium repletion, the concentration of this protein increased in parallel with the final steps in aldosterone biosynthesis, as reflected by increased production of 18-hydroxycorticosterone (18-OH-B) and aldosterone (Aldo) and decreased outputs of deoxycorticosterone (DOC), corticosterone (B), and 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) by incubated capsular adrenals. Sodium restriction of rats also resulted in a marked increase in the concentration of the same mitochondrial protein.⁸ This protein crossreacted with a monoclonal antibody raised against purified bovine adrenal cytochrome P-450_{11 β} .⁹ The same antibody stained a protein band with a molecular weight of 51,000 in mitochondria of zona fasciculata cells and in mitochondria of zona glomerulosa cells of rats in which aldosterone biosynthesis had been suppressed by potassium restriction and sodium loading.

ENZYMOLGY OF THE FINAL STEPS OF ALDOSTERONE BIOSYNTHESIS

There is long-standing evidence that the final two steps of aldosterone biosynthesis are catalyzed by mitochondrial cytochrome P-450. However, the identity of this enzyme has only recently been elucidated. Two different groups of investigators have shown that preparations of bovine or porcine cytochrome P-450_{11 β} , removed from mitochondria and purified to electrophoretic homogeneity, catalyze not only 11 β -hydroxylation, but also the two-step conversion of B to Aldo, irrespective of the zonal origin of the reconstituted enzyme.^{10,11} If all three steps involved in the conversion of

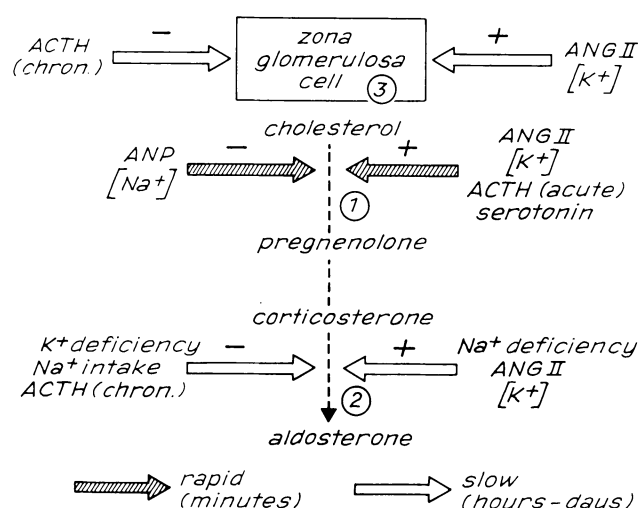


FIGURE 1. Multifactorial regulation of aldosterone biosynthesis at three different levels: 1) acute stimulation or inhibition of early biosynthetic steps; 2) long-term stimulation or suppression of late biosynthetic steps; and 3) growth or involution of zona glomerulosa cell. ANG II, angiotensin II; ANP, atrial natriuretic peptides. From Müller J (1988).¹

DOC to Aldo are mediated by the same enzyme ("one-enzyme" hypothesis; reference 9), the variable rates of corticosterone methyl oxidations 1 and 2 in adaptation to the sodium and potassium status are possibly regulated by unknown mitochondrial factors. Local inhibitors might also be responsible for the constant suppression of the inherent aldosterone biosynthetic activity of cytochrome P-450_{11β} in zona fasciculata mitochondria in the bovine or porcine adrenal cortex. However, neither the "one-enzyme" hypothesis nor a previously postulated "two-enzyme" hypothesis⁹ are valid for the rat species, according to recent evidence obtained in our laboratory¹² and by other investigators.¹³ The immunoreactive 51K protein was isolated from zona fasciculata mitochondria and purified to electrophoretic homogeneity by chromatography on octyl-Sepharose. In a reconstituted enzyme system (with bovine adrenal adrenodoxin and adrenodoxin reductase), it converted DOC to B and 18-OH-DOC but not to 18-OH-B and Aldo. The immunoreactive 49K protein was isolated from zona glomerulosa mitochondria of rats kept on a low-sodium, high-potassium regimen. By chromatography on octyl-Sepharose, it could be separated from the 51K protein but not from other mitochondrial proteins. A reconstituted eluate fraction containing the 49K protein converted DOC to B, 18-OH-DOC, 18-OH-B, and Aldo. These findings indicate that the rat adrenal cortex contains two different forms of active cytochrome P-450_{11β} (51K and 49K), with both of them capable of monohydroxylating DOC in either the 11β- or the 18-position, but with only one of them (the 49K form) also capable of catalyzing the conversions of B to 18-OH-B and Aldo ("one enzyme, two forms" hypothesis; reference 9). The appearance of the 49K form of the enzyme in zona glomerulosa mitochondria is a crucial element in the adaptation of aldosterone biosynthesis to sodium deficiency or potassium intake in rat. An enzyme comparable to the 49K form of cytochrome P-450_{11β} has as yet not been discovered in human adrenals or adrenals from any animal species other than the rat.^{11,14} The elucidation of the mechanisms of zonal specificity and long-term adaptation of aldosterone biosynthesis to sodium and potassium intake in these organisms remains a continual challenge for present and future investigators.

ACKNOWLEDGMENTS

The expert technical assistance of Heidi Seiler, Carmen Matt, Gaby Haesler-Vetsch, and Dora Schmid is gratefully acknowledged.

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