Aldosterone biosynthesis in the human adrenal cortex and associated disorders

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A R T I C L E   I N F O

A B S T R A C T

Aldosterone is one of the mineralocorticoids synthesized and secreted by the adrenal glands, and it plays pivotal roles in regulating extracellular fluid volume and blood pressure. Autonomous excessive aldosterone secretion resulting from adrenocortical diseases is known as primary aldosteronism, and it constitutes one of the most frequent causes of secondary hypertension. Therefore, it is important to understand the molecular mechanisms of aldosterone synthesis in both normal and pathological adrenal tissues. Various factors have been suggested to be involved in regulation of aldosterone biosynthesis, and several adrenocortical cell lines have been developed for use as *in vitro* models of adrenal aldosterone-producing cells, for analysis of the underlying molecular mechanisms. In this review, we summarize the available reports on the regulation of aldosterone biosynthesis in the normal adrenal cortex, in associated disorders, and in *in vitro* models.

1. Introduction

Aldosterone is known to regulate extracellular fluid volume and potassium exchange; it is synthesized in the zona glomerulosa (ZG) of the normal adrenal gland, primarily in response to angiotensin II and serum potassium, resulting in subsequent depolarization and opening of voltage-activated Ca^{2+}-channels, with activation of the calcium signaling pathway [1]. The classic pathway of aldosterone action involves binding to cytosolic mineralocorticoid receptors (MR) and subsequent translocation to the nucleus, followed by transcription and translation of effector proteins involved in regulating the sodium–potassium balance across renal tubular epithelial cells [2,3].

Primary aldosteronism (PA) has been reported to be present in approximately 5–20% of all patients with hypertension [4–6]. In addition, patients with PA are known to show a significantly higher incidence of cardiovascular events than hypertensive subjects [7]. Aldosterone-producing adenoma (APA) and idiopathic hyper-aldosteronism (IHA) are two principal causes of PA, both characterized by autonomous aldosterone production from pathological adrenal tissues. In this review, we will focus on the proposed molecular mechanisms of aldosterone biosynthesis in both normal and pathological adrenocortical tissues.

2. The expression of aldosterone-producing enzymes in normal human adrenal glands

The adult human adrenal cortex can be divided, histologically speaking, into three different areas; the zona glomerulosa (ZG), the zona fasciculata (ZF), and the zona reticularis (ZR) [8]. Each zone is known to secret different types of adrenocortical steroid hormones: mineralocorticoids in the ZG, glucocorticoids in the ZF, and adrenal androgens in the ZR. However, the functional zonation of the adrenal cortex remains controversial. 11-β-Hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) are regarded as zone-specific steroidogenic enzymes that are involved in the final steps of biosynthesis of cortisol and aldosterone, respectively. Cytochrome P450scc (CYP11A), 3β-hydroxysteroid dehydrogenase (HSD3B), and cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2) are all expressed both in the ZG and the ZF. Adrenocortical cells positive for CYP11B2 and negative for CYP17 are functionally classified as mineralocorticoid-producing cells, whereas cells positive for CYP11B1 and negative for CYP17 are regarded as glucocorticoid-producing cells. Gomez-Sanchez et al. recently reported the development of novel and specific monoclonal antibodies against human CYP11B1 and CYP11B2 [9]. They demonstrated that CYP11B2 immunoreactive

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cells can be classified into two categories: those scattered in the ZG, and those forming tight clusters [9]. They have also reported that CYP11B1 immunoreactive cells are abundant mainly in the area corresponding to the ZF, and that CYP11B1-immunoreactive cells extend up to the capsule in many parts of the normal human adrenal cortex [9].

3. Aldosterone production in human fetal adrenal gland

In human fetuses, the three layers of the adrenal cortex become morphologically discernible during the final trimester of pregnancy, i.e., the definitive zone (DZ), the transitional zone (TZ), and the fetal zone (FZ). The FZ is the inner zone, in which CYP11A1, CYP17, and sulfokinase are expressed, resulting in the production of DHEAS. The TZ is located between the DZ and the FZ; CYP11A1, CYP17, CYP21A2, and CYP11B1 are expressed in the TZ for cortisol production. The DZ is the outermost zone, and is considered to be the progenitor of the adult adrenal cortex, with the potential to produce mineralocorticoids [10–12]. The expression patterns of steroidogenic enzymes in fetal adrenocortical cells, especially HSD3B and CYP17, indicate that both HSD3B- and CYP17-positive cortical cells preferentially produce cortisol, that HSD3B-positive and CYP17-negative cells preferentially produce mineralocorticoids, and HSD3B-negative and CYP17-positive cells preferentially produce DHEAS. The expression profiles of steroidogenic enzymes in each individual gestational term are summarized in Table 1 [12].

In humans and rhesus monkeys, biosynthesis of glucocorticoids in fetal adrenal glands usually begins at an early stage of gestation [13] and the mineralocorticoid biosynthesis pathway is confined to the definitive zone of the primate fetal adrenal cortex in the third trimester (weeks 18–21) [14]. The biosynthesis of mineralocorticoids requires the expression of CYP11A1, HSD3B type 2 (HSD3B2), CYP11B2, and CYP21A2. Coulter et al. reported the results of an immunohistochemical analysis of the expression profiles of mineralocorticoid synthases in the fetal adrenal cortex [15]. In the early stages of human gestation, CYP21A2 is expressed in several cell islands throughout the DZ, the TZ, and the FZ, but not in the adrenomedullary cells [15]. During weeks 13–24 of gestation, CYP11B1/B2 is present in the TZ and the FZ, but not in the DZ or the adrenomedullary cells. In rhesus monkeys, however, CYP21A2 is expressed more abundantly in the DZ and the TZ than the FZ, and all cells of the TZ and the FZ, but not the DZ, are positive for CYP11B1/B2 staining [15].

The above results all indicate that mineralocorticoid synthesis in the human fetal adrenal glands might begin near term, because the enzymes required for synthesis, such as CYP21A2 and CYP11B2,

are usually not expressed in the DZ in the early stages of gestation. Late in gestation, CYP11A1, CYP17, HSD3B2, CYP21A2, CYP11B1, and CYP11B2 are all expressed in the DZ [15]. In addition, CYP17 is not usually expressed in the DZ during gestation. Therefore, the DZ could represent the progenitor cells of the ZG of the adult adrenal cortex, but further investigation is required for clarification. In fetuses, approximately 80% of the mineralocorticoids in the blood are derived from the fetal adrenal cortex [16,17]. Near week 16 of gestation, angiotensin II (AT) receptors are expressed in fetal adrenal cortical cells. The AT type 2 receptor is expressed in the FZ, whereas the AT type 1 receptor is expressed in both the FZ and the DZ [18]; however, the biological significance of the differential expression profiles of these receptors remains unknown.

In a previous study, aging-related impairment of aldosterone production in the ZG was reported in rats [19]. The plasma concentration of aldosterone was lower in old rats (24 months) than in young rats (3 weeks). In addition, the activity of the aldosterone-producing precursor steroidogenic enzyme and the responsiveness to Ang II, foskolin, and ACTH was lower in old rats. Therefore, the ability to convert steroid precursors to aldosterone declined with aging in rats [19]. Aiba and Fujibayashi summarized the results of immunohistochemical analyses of human resection and autopsy cases, and reported on the aging-related development of the ZG [20]. In newborns to individuals in their third decade, the ZG is well-developed and localized in subcapsular lesions. However, in individuals beyond their fourth decade, the ZG is replaced by lesions known as progenitor zones (ZPs), which express HSD3Bs instead of CYP11B2 [20]. Therefore, adrenocortical remodeling occurs with aging by subcapsular localization of the ZP, which has the potential for bidirectional differentiation, into ZG or ZF, owing to secondary aldosteronism under conditions of high Na, low K, and severe stress [20].

4. The status of aldosterone-producing enzymes in PA

The mechanisms causing the autonomous production of excessive aldosterone in APA have not been fully elucidated at this juncture. However, several studies have indicated that overproduction of steroid hormones in adrenocortical tumors was primarily caused by the disordered and/or disorganized expression of steroidogenic enzymes. APA tissues in general express relatively high levels of CYP11B2, but StAR mRNA expression is usually not elevated in these tumor tissues [21]. We have recently demonstrated the immunoreactivity of CYP11B2 in cases of APA, using the specific antibodies described above [22,23]. In these studies, CYP11B2 immunoreactivity in APA was weak and heterogeneous (Fig. 1A), and the percentage and relative intensity of CYP11B2 immunoreactivity in APA were not necessarily significantly higher than those in the ZG in normal adrenal glands (NA) [22,23]. Therefore, the relative immunointensity and percentage of CYP11B2 immunoreactivity may not necessarily reflect the status of overproduction of aldosterone in APA. CYP11B1 and CYP17 have been reported to be present in APA tumor cells (Fig. 1B and C) [22,23]. In addition, we have also demonstrated that CYP11B1 and CYP17 were co-localized in the great majority of APA tumor cells [22,23] which indicated that these same APA tumor cells could secrete cortisol as well as aldosterone [24,25]. Nanba et al. sub-classified APA into three groups based on semi-quantitative analysis of protein expression: CYP11B2-dominant, CYP11B2/CYP11B1-equivalent, and CYP11B1-dominant groups [26]. They reported that the CYP11B2/CYP11B1-equivalent and CYP11B1-dominant groups had the potential to produce cortisol autonomously and that the CYP11B2-dominant group showed a potential association with lower serum potassium through mineralocorticoid action [26]. Under normal conditions, CYP11B2 is localized to the ZG, and CYP11B1 is localized exclusively

Table 1
Expression profiles of steroidogenic enzymes in each individual gestational term [12].

Middle gestation			
Steroidgenic enzymes	DZ	TZ	FZ
P450 scc	+	+	+
CYP17	–	++	+
CYP21A2	++	++	+
HSD3B	–	–	–
CYP11B1/B2	–	+	+

Late gestation			
Steroidgenic enzymes	DZ	TZ	FZ
P450 scc	+	+	+
CYP17	–	++	+
CYP21A2	++	++	+
HSD3B	++	+	–
CYP11B1/B2	+	++	+