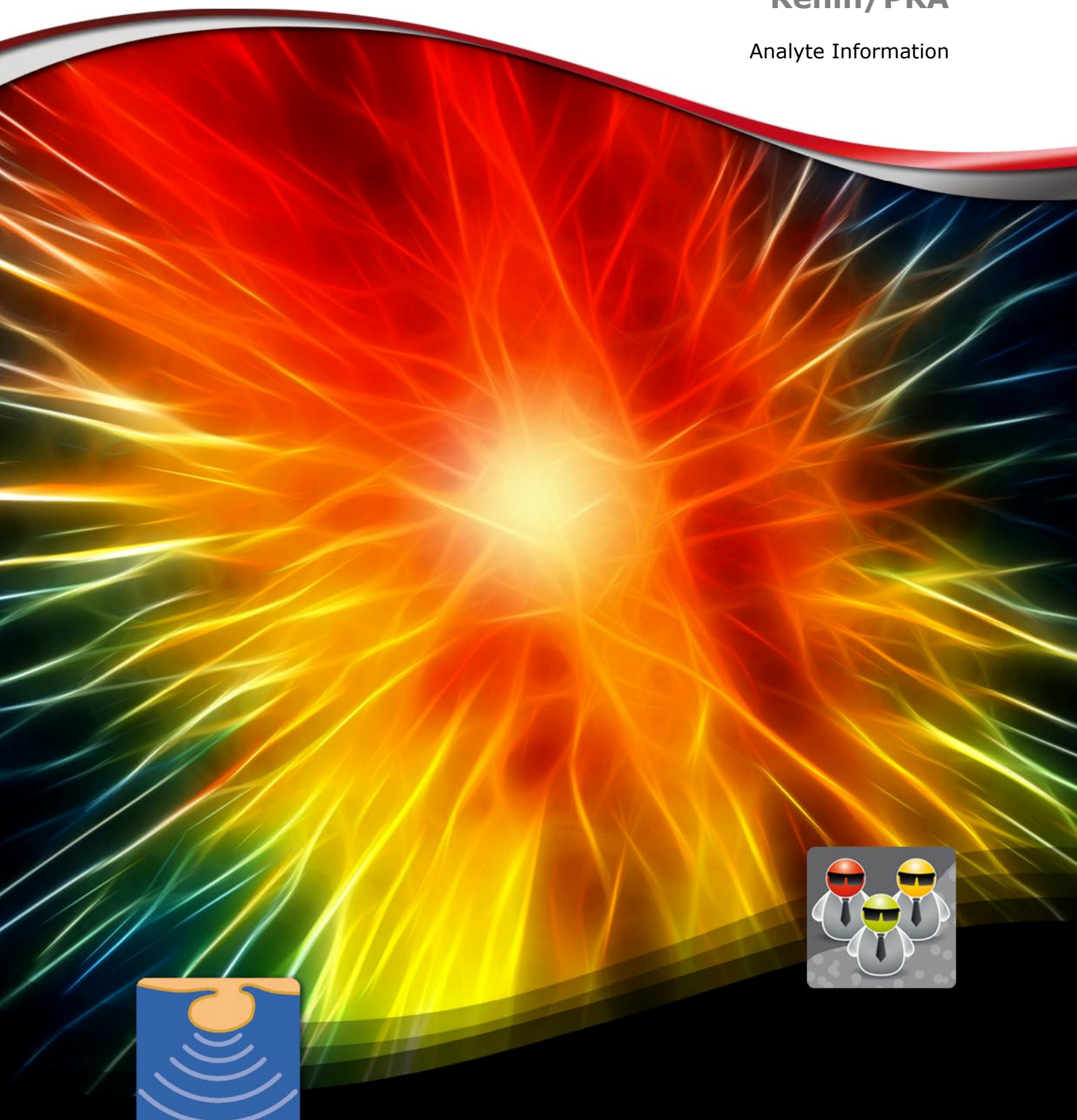




# Adrenal Function

Renin/PRA

Analyte Information



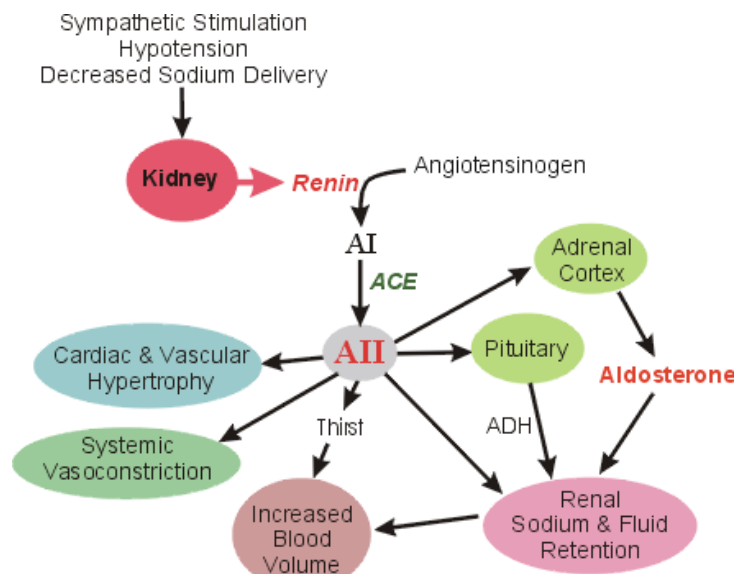


## Renin/Plasma Renin Activity (PRA)

### Introduction

Renin is an enzyme that plays an important role in the body's mineral, salt and water (fluid) balance. This enzyme participates in the body's renin-angiotensin-aldosterone system (RAS). RAS mediates extracellular volume (i.e. that of blood plasma, lymph and interstitial fluid) and in arterial vasoconstriction, and thus regulates blood pressure.

**Fig. 1:** Contextualization of renin into the RAS (Renin-Angiotensin-Aldosterone system) and the production of Angiotensin II, which has been found to be the major factor influencing aldosterone secretion by the adrenal glands and thus plays a key role in several forms of hypertension.



*A I – Angiotensin I*  
*A II – Angiotensin II*

*ACE – Angiotensin Converting Enzyme*  
*ADH – Anti-Diuretic Hormone*

Renin is a proteolytic enzyme belonging to the family of aspartyl proteases<sup>1,2,3</sup>. It is a glycoprotein with a molecular weight of approximately 40 kDa that catalyzes cleavage of protein angiotensinogen in order to generate ten amino acid peptide angiotensin I<sup>4</sup> in the so-called Renin-Angiotensin-Aldosterone System (RAS or RAAS).



This enzyme is also known as

- Angiotensinogenase
- Angiotensin-forming enzyme

### **Biochemistry and Metabolism**

The enzyme is synthesized as inactive prorenin in the juxtaglomerular cells of the kidney and released into the blood in an active state in response to various metabolic stimuli.

Prorenin is activated to renin enzymatically (callicrein, cathepsin B), through exposure to acid (acid activation) or by a cold (cryoactivation) environment.

The half-life of active renin is up to 80 minutes. As the active renin is not stored in the body, it has to be produced nearly continuously. If an increase of renin is needed, it becomes necessary to increase the production of (pre)prorenin in the transcription step<sup>5</sup>. The change in the transcription rate is a complex mechanism and takes some time – from minutes to tens of minutes and the stimuli must continue for a sufficiently long time. Hence the RAS is able to serve as a medium-term blood-pressure regulation mechanism, but not as an immediate or short-term response.

### **Physiological Function**

The renin-angiotensin-aldosterone system (RAS or RAAS) plays an important role in regulating blood volume and systemic vascular resistance, which together influence cardiac output and arterial pressure. As the name implies, there are three important components in this system:

- Renin
- Angiotensin
- Aldosterone

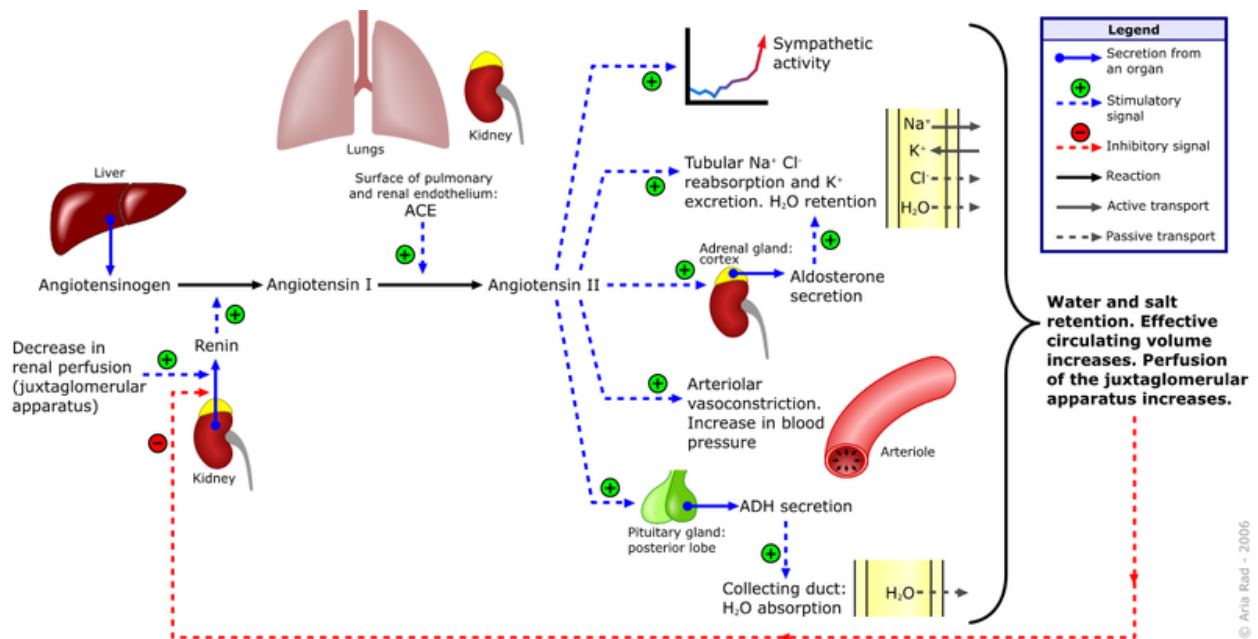
When blood volume is low or when a drop in blood pressure occurs, the kidneys secrete renin. The enzyme circulates in the bloodstream and cleaves (hydrolyzes) angiotensinogen secreted from the liver into the peptide angiotensin I.

Angiotensin I is further cleaved in the lungs by endothelial-bound angiotensin converting enzyme (ACE) into angiotensin II, the most vasoactive peptide<sup>6</sup>.



Angiotensin II is a potent constrictor of all blood vessels. It acts on the musculature and thereby raises the resistance posed by these arteries to the heart. The heart, trying to overcome this increase in 'load', works more vigorously, causing blood pressure to rise. Angiotensin II also acts on the adrenal glands and releases aldosterone, which stimulates the epithelial cells of the kidneys to increase re-absorption of sodium, chloride and water, increasing blood volume and blood pressure. The RAS also acts on the CNS (central nervous system) to increase water intake by stimulating thirst, and to conserve blood volume by reducing urinary loss through the secretion of vasopressin (Antidiuretic Hormone – ADH) from the posterior pituitary gland.

**Fig. 2: Renin-Angiotensin-Aldosterone System**



The renin-angiotensin-aldosterone system (RAS).

Start reading this schema from the left, at the point labeled "Decrease in renal perfusion (juxtaglomerular apparatus)". Alternatively, the RAS can also be activated by a low NaCl concentration in the macula densa or by sympathetic activation. Blue and red dashed arrows indicate stimulatory or inhibitory signals, respectively, which are also indicated by +/- . In the tubule and collecting duct graphics, the grey dashed arrows indicate passive transport processes, contrary to the active transport processes which are indicated by the solid grey arrows. The other solid arrows indicate either a secretion from an organ (blue, with a starting spot) or a reaction (black). These two processes can be stimulated or inhibited by other factors.

Source: A.Rad, Wikipedia, 2006

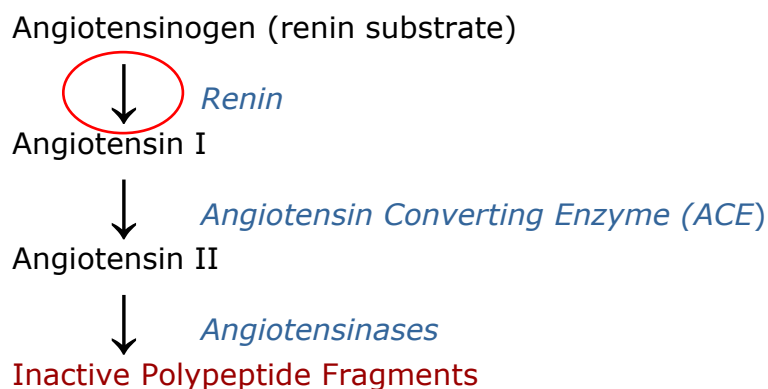


If the renin-angiotensin-aldosterone system is too active, blood pressure becomes too high. There are many drugs which interrupt different steps in this system to lower blood pressure. Such drugs help to control high blood pressure (hypertension), heart failure, kidney failure, and the harmful effects of diabetes<sup>7</sup>.

### **The measurement of plasma renin activity, or directly the circulating renin: A reliable approach for RAS assessment**

As mentioned above, Angiotensin II is the most potent vasopressive hormone in the body, and plays a key role in the regulation of systemic blood pressure. It is the physiological end-point of RAS, and thus it seems to be good biomarker for the efficacy of this system. However, the rapid degradation of Angiotensin II in vivo precludes its analytical use<sup>8</sup>.

### **The Renin-Angiotensin cascade**



Since angiotensin I and II levels are directly related to renin activity, assessment of the system can be evaluated either through measurement of plasma renin activity (i.e. rate of conversion of angiotensinogen to angiotensin I) or through direct measurement of renin concentration.

### **Plasma renin activity or direct renin determination?**

There are advantages and disadvantages of both approaches.



## **PRA**

PRA determination is based on the rate of production of angiotensin I. PRA is expressed as the amount (in ng) of angiotensin I, generated within one hour in 1 mL of plasma (ng/mL/hr).

It is obtained by the determination of angiotensin I after incubation of plasma at 37°C together with its substrate present in plasma - endogenous angiotensinogen, in presence of inhibitors of ACE (Angiotensin Converting Enzyme) and then subtracting the amount of baseline angiotensin I in a control plasma aliquot kept at 4°C.

Plasma renin activity is well established determination, and its results are published in many studies. It also seems to reflect better biological activity of RAS.

On the other hand, the results may be, in rare cases, affected by low concentration of angiotensinogen substrate. Also, the whole determination is more laborious comparing to the determination of renin concentration. There is an additional enzymatic reaction that precedes the immunoassay. There is also rather unclear situation concerning the collection of blood. It is generally considered that blood has to be chilled immediately after collection and processed cooled till the controlled enzymatic creation of Angiotensin I begins or till plasma is frozen. The reason is to prevent from continual creation of angiotensin I and to avoid depletion of angiotensinogen. But there is also a contrary opinion suggesting that blood should be collected and processed at room temperature, to avoid cryoactivation of renin with subsequent increase of PRA.

## **Renin**


This method uses direct measurement of renin mass by means of "sandwich" immunoassay. Collection and processing is performed in room temperature processed at room temperature, to avoid cryoactivation of renin.




## Levels

The determination of renin/PRA levels is used in the diagnosis and follow-up of various disorders. Descriptions of these items are listed below under the heading "Diagnostic utility". In addition, there are a large number of factors and conditions influencing renin and angiotensin production and secretion. They are summarized in the following tables:

Factors stimulating the production and/or activity of renin<sup>9,10</sup>:

- 
- bleeding
  - upright posture
  - dehydration
  - heart failure
  - all other factors activating SANS (sympathetic nervous system), including mental stimuli
  - circulating catecholamines
  - sodium depletion
  - kalium depletion
  - diuretics
  - constriction of renal artery or aorta
  - ATI (Angiotensin I) receptor blockers
  - ACE (Angiotensin converting enzyme) inhibitors
  - fluid loss from gastrointestinal tract
  - nephrotic syndrome
  - direct vasodilatory therapy
  - hypoglycemia
  - increased level of thyroid gland hormones, particularly hyperthyroidism
  - sympathomimetics
  - acute glomerulonephritis
  - gestation
  - in women before ovulation and during luteal phase

Factors inhibiting the production and/or activity of renin<sup>11</sup>:

- 
- advanced age
  - decrease of sympaticus activity
  - increased concentration of sodium and kalium ions in the macula densa
  - increase of plasmatic kalium concentration
  - vasopressin
  - blockers of beta-1-adrenergic receptors
  - substances with central sympatholytic effect (some renin inhibitors)
  - angiotensin II
  - aldosterone
  - atrial natriuretic peptides,
  - non-steroid antirheumatics
  - autonomous neuropathy
  - oral contraceptives containing estrogens
  - diabetes mellitus
  - ingestion of exogenous mineralocorticoids
  - in women during follicular phase



Sample reference intervals for renin levels<sup>12</sup> and plasma renin activity<sup>15</sup> are listed in the following table. These are strictly for informational purposes, as appropriate reference levels vary according the assay used.

For each assay, the relevant reference values are shown in the appropriate Instructions for Use (IFU).

#### **Table 1 – Typical renin levels**

<b>Specimen</b> (plasma)	<b>Reference interval</b> (pg/mL)
Normal healthy adults (n = 84)	5.0 – 27.8

#### **Table 2 – Typical PRA levels**

<b>Specimen</b>	<b>Reference interval</b> (ng/mL/hod)
Cord blood:	4 - 32
Newborns (1-7 days):	2 - 35
Children	
1-12 months:	2.4 - 37
1-3 years	1.7 - 11.2
3-5 years	1.0 - 6.5
5-10 years	0.5 - 5.9
10-15 years	0.5 - 3.3
Adults	
(supine):	0.2 - 1.6
(upright – 4 hrs):	0.7 - 3.3





## **Diagnostic utility – clinical significance of renin and PRA determination**

The symptom most closely connected with renin production and the Renin–Angiotensin–Aldosterone–System (RAS) is hypertension. In hypokalemic states the determination of RAS activity is of great importance as well.

Endocrine hypertension frequently occurs in cases of primary disorders of adrenal gland (pheochromocytoma, primary hyperaldosteronism, deoxycorticosterone or cortisol producing tumors), pituitary gland tumors producing adrenocorticotrophic hormone (ACTH) and renal disorders (renin producing tumors, renovascular disease, and some types of tubulopathies).

Arterial hypertension can occur in cases of endocrine disorders (acromegaly, thyreotoxicosis, hypothyreosis, hyperparathyreosis, hyperinsulinism).

### **Mineralocorticoid hypertension**

Mineralocorticoid hypertension is hypertension due to the effects of mineralocorticoid hormones (mainly aldosterone). Mechanisms by which these hormones lead to hypertension include:

- Primary excess of mineralocorticoids
- Syndroms with secondary excess of mineralocorticoids
- Other hypertension syndromes with pseudohyperaldosteronism

### **Primary excess of mineralocorticoids**

The disorders are characterized by hypertension, hypokalemia, RAS suppression and normal or subnormal cortisol production. The biochemical findings for appropriate markers connected with the disorders of interest are summarized in Table 1<sup>13</sup>.



**Table 1: Primary hypermineralocorticoidism - Biochemical findings**

	Plasma Na+	Plasma K+	ALD	Other MCH	Renin/PRA	Cort	ACTH	Hematocrit	BUN/CR
Adenom producing ALD	N/↑	↓	↑↑	↑	↓↓	N	N	↓	N
Carcinom producing ALD	↑	↓	↑↑	↑↑	↓↓	N/↑	N/↓	↓	N
Hyperplasia:									
Idiopathic hyperaldosteronism	N	↓	↑	N	↓	N	N	N/↓	N
Undetermined hyperaldosteronism	N	N/↓	↑	N	↓	N	N	N/↓	N
Hyperaldosteronism curable by glucocorticoids	N	↓	↑	N	↓	N	N	N/↓	N
Primary hyperplasia	N/↑	↓	↑↑	↑	↓↓	N	N	↓	N
Defective 11β-hydroxylase	N	N/↓	↓	↑	N/↓	N/↓	↑	N/↓	N
Defective 17α-hydroxylase	N/↑	↓	↓	↑↑	↓↓	↓	↑	N/↓	N

ALD	Aldosterone	↑	Increase
MCH	Mineralocorticoid hormones	↓	Decrease
Cort	Cortisol	↑↑	Significant increase
ACTH	Adrenocorticotrophic hormone	↓↓	Significant decrease
BUN	Plasmatic urea nitrogen	N	Normal
CR	Creatinin		

### Syndroms with secondary excess of mineralocorticoids

The activation of the zona glomerulosa of the adrenal gland due to RAS activity is associated with various states characterized by aldosterone overproduction, e.g., renal disorders connected with salt loss, dehydration, cirrhosis, heart failure and decrease of intravascular volume, and overusage of laxatives or diuretics. These cases are rarely accompanied by hypertension. In contrast hypertension is typical for disorders with direct renin overproduction, e.g., renovascular hypertension and renin-producing tumors. The biochemical findings for appropriate markers connected with the disorders of interest are summarised in Table 2<sup>13</sup>.



**Table 2: Secondary hypermineralocorticoidism - Biochemical findings**

	Plasma Na <sup>+</sup>	Plasma K <sup>+</sup>	ALD	Other MCH	Renin/PRA	Cort	ACTH	Hemato-crit	BUN/CR
Renovascular hypertension	<b>N/↑</b>	<b>N/↓</b>	<b>↑</b>	<b>N</b>	<b>↑</b>	<b>N</b>	<b>N</b>	<b>↑</b>	<b>N/↑</b>
Cancer producing renin	<b>N/↑</b>	<b>↓</b>	<b>↑↑</b>	<b>N</b>	<b>↑↑</b>	<b>N</b>	<b>N</b>	<b>↑</b>	<b>N</b>
Accelerated hypertension	<b>N</b>	<b>N/↓</b>	<b>↑</b>	<b>N</b>	<b>↑</b>	<b>N</b>	<b>N</b>	<b>N/↑</b>	<b>N/↑</b>
Estrogen treatment	<b>N</b>	<b>N/↓</b>	<b>↑</b>	<b>N</b>	<b>N/↑</b>	<b>N/↑</b>	<b>N</b>	<b>N/↓</b>	<b>N</b>
Cushing's syndrome	<b>N</b>	<b>N/↓</b>	<b>N/↓</b>		<b>N</b>	<b>↑</b>	<b>N/↑</b>	<b>N</b>	<b>N</b>

ALD	Aldosterone	↑	Increase
MCH	Mineralocorticoid hormones	↓	Decrease
Cort	Cortisol	↑↑	Significant increase
ACTH	Adrenocorticotropic hormone	↓↓	Significant decrease
BUN	Plasmatic urea nitrogen	N	Normal
CR	Creatinin		

### Other hypertension syndromes with pseudohyperaldosteronism

Secondary mineralocorticoid deficiency (pseudohyperaldosteronism) comprises a heterogeneous group of disorders with abnormally low mineralocorticoid production due to suppressed renin production.

Hypertension, hypokalemia and metabolic alkalosis are the usual clinical manifestations of decreased renin production. This is due to increased sodium retention and by volume expansion caused by exogenous mineralocorticoids, mineralocorticoid-like substances or by renal tubular defect.

If renin production is suppressed and thus insufficient to stimulate production of mineralocorticoids, sodium loss, hyperkalemia and metabolic acidosis occur.

The biochemical findings for appropriate markers connected with the disorders of interest are summarised in Table 3<sup>13</sup>.



**Table 3: Pseudohyperaldosteronism - Biochemical findings**

	Plasma Na <sup>+</sup>	Plasma K <sup>+</sup>	ALD	Other MCH	Renin	Cort	ACTH	Hema-tocrit	BUN/CR
Pseudohyperaldosteronism (Liddle's syndrome)	N/↑	↓	↓	N/↓	↓	N	N	↓	N
Arnold-Healy-Gordon's syndrome	N/↑	↑	↓	N/↓	↓	N	N	N/↓	N
Treatment with synthetic MCH	N/↑	↓	↓	N/↓	↓	N	N	↓	N
Licorice digestion	N/↑	↓	↓	N/↓	↓	N	N	↓	N
Primary cortisol resistance	N/↑	↓	N/↓	↑	N/↓	↑	↑	N/↓	N

ALD	Aldosterone	↑	Increase
MCH	Mineralocorticoid hormones	↓	Decrease
Cort	Cortisol	↑↑	Significant increase
ACTH	Adrenocorticotrop hormone	↓↓	Significant decrease
BUN	Plasmatic urea nitrogen	N	Normal
CR	Creatinin		

## Diagnostic utility – Summary of practical applications

### When to measure renin or PRA?<sup>14</sup>

In cases of one or more of the following situations:

- Diastolic blood exceeds 110 mm Hg (hypertension of renal origin)
- Hypokalemia: <3.8 mmol/L (secondary hyperaldosteronism, primary mineralocorticism)
- Insufficient response to antihypertensive treatment
- Determination of functional character of a renal artery stenosis (measurement of renin in renal veins during acute inhibition of the converting enzyme)
- Increased blood pressure linked with cancer (ectopic production of renin)



## Renin/PRA values and healthy status – overview<sup>15</sup>

### ↑ RENIN/PRA INCREASE

*With consequent secondary aldosteronism*

Hypertensive states:

- malignant or severe hypertension
- unilateral renal disease with malignant or severe hypertension
- renal parenchymal diseases
- renin-secreting tumors
- oral contraceptive-induced hypertension
- pheochromocytoma

Edematous normotensive states

- cirrhosis
- hepatitis
- nephrosis
- congestive heart failure

Hypokalemic normotensive states

- Bartter's syndrome (Juxtaglomerular cell hyperplasia)
- other nephropathies with Na<sup>+</sup> or K<sup>+</sup> wastage
- alimentary disorders with electrolyte loss

*Without consequent secondary aldosteronism*

- adrenocortical insufficiency
- potassium depletion state (alimentary)

### ↓ RENIN/PRA DECREASE

*With adrenocortical disease*

Hypertensive states:

- primary aldosteronism due to adrenal carcinoma
- pseudoprimary or idiopathic aldosteronism (usually bilateral adrenocortical hyperplasia)
- glucocorticoid-suppressible aldosteronism
- adrenal carcinoma with mineralocorticoid excess
- adrenal enzyme defects with oversecretion of other mineralocorticoids

*Without adrenocortical disease*

Hypertensive states:

- low-renin essential hypertension
- in certain patients with renal parenchymal disease
- Liddle's syndrome (pseudohyperaldosteronism)
- licorice or mineralocorticoids ingestion

Normotensive states:

- renal parenchymal diseases
- autonomic disorders with postural hypotension
- uninephrectomized subjects
- drug-induced adrenergic blockade



## References

- 1 Miller R.P., Poper C.J., Wilson C.W. and DeVito E.: Renin inhibition by pepstatin. *Biochem. Pharmacol.* 1972, 21, 2941-2944.
- 2 Hobart P.M., Fogliano M.O., O'Coner B.A., Schaefer I.M., and Chirgwin, J.M.: Human renin gene structure and sequence analysis. *Proc. Natl. Acad. Sci. USA* 1984, 81, 5026.
- 3 Murakami, K., Hirose, S., Miyazaki, H., Imai, T., Hori, H., Hayashi, T., Kageyama, R., Ohkuda, H., and Nakanishi, S.: Complementary DNA sequences of renin. State of the art review. *Hypertension* 1984, 6, 195.
- 4 Dorland 's Illustrated Medical Dictionary. 30th ed. Philadelphia: WB Saunders, 2003.
- 5 Toffelmire E.B., Skater K., Corvol P., et al: Response of plasma prorenin and active renin to chronic and acute alterations of renin secretion in normal humans. *J. Clin. Invest.* 1989, 83, 679-687.
- 6 Fujino T., Nakagawa N., Yuhki K., et al.: Decreased susceptibility to renovascular hypertension in mice lacking the prostaglandin I<sub>2</sub> receptor IP. *J. Clin. Invest.* 2004, 114 (6), 805-812.
- 7 High Blood Pressure: Heart and Blood Vessel Disorders. Merck Manual Home Edition, Solomon, Scott D; Anavekar, Nagesh: A Brief Overview of Inhibition of the Renin-Angiotensin System: Emphasis on Blockade of the Angiotensin II Type-1 Receptor. *Medscape Cardiology* 2005, 9 (2).
- 8 Sealey, J.E.: Plasma renin Activity and Plasma Prorenin Assays. *Clin. Chem.* 1991, 37/10(B): 1811-1819.
- 9 Ganong WF: review of medical Physiology, 19th ed. Stamford, Appleton & Lange, A Simon Schuster Company, 1999, 433-446.
- 10 Deinum J., Schalekamp M.A.D.H.: Renin and Prorenin. In Oparil S, Weber MA (eds): *Hypertension: a companion to Brenner & rector 's kidney*. Philadelphia, W.B. Saunders Company, 1999, 70-76.
- 11 Kára T, Souček M: Autonomní nervový systém. In Souček M, Kára T a kol. *Klinická patofyziologie hypertenze*. Praha: Grada Publishing, 2002.
- 12 Renin IRMA DSL25100, Instructions for Use, BeckmanCoulter, 2009.
- 13 Greenspan F.S., Baxter J.D.: *Basic & Clinical Endocrinology (Czech 1st ed)*, 2003, Nakladatelství H&K Vysehradská.
- 14 Demers L.M.: The Adrenal Cortex. In Burtis CA, Ashwood ER, Bruns DE, editors: *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th edition. St.Louis, Elsevier Inc, 2006, 2003-2033.
- 15 Wu Alan HB Wu: *General Clinical Tests*. In *Tietz Clinical Guide to Laboratory Tests*. St.Louis, 2006, WB Saunders Company, 1967-2002