

50th Anniversary of Aldosterone

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This year marks the 50th anniversary of Simpson and Tait's isolation of electrocortin (aldosterone) (Fig. 1). Five decades of research have yielded extensive insight into the biological actions mediated through this once elusive molecule, in particular, areas of sodium and potassium metabolism and the kidney. The history of discovery surrounding the mechanisms of regulation and action of aldosterone in the 1960s and 1970s provides a unique view of the birth of molecular biology and its consequent impact on the field of genomic research in the 1980s, 1990s, and the 21st century. Interestingly, recent areas of study have focused on the non-genomic effects of aldosterone and its involvement in inflammation and fibrosis in cardiovascular diseases. This has taken what might have at one time been considered a steroid hormone with a narrow pathological consequence into the realm of influencing widely prevalent diseases such as nephropathy, cardiomyopathy, and vasculopathy.

Here, we provide a historic review of the discovery of aldosterone, emphasizing its widely expanding involvement in human disease. We begin describing the early years of aldosterone discovery including mention of its involvement in fibrosis by Selye. Next, we explore how molecular biology and genetics yield novel mechanisms of action and regulation. Finally, we revisit the area of nonclassical effects and the expanding involvement of aldosterone in common cardiovascular diseases.

The early years

The earliest recorded mention of the adrenal gland is Bartolomeo Eustacchio's 1563 copper-etched depiction of "glandulae Renibus incumentes" (1). Several centuries later, Thomas Addison (1849) published his description of a lethal clinical syndrome resulting from destruction of the adrenal glands (2), thereby providing the stimulus for subsequent modern physiological investigations of the adrenal cortex. During the early part of the 20th century, focus turned to the role the adrenal cortex played in carbohydrate metabolism. By the late 1930s, most of the glucocorticoids produced by the adrenal cortex had been isolated and structures characterized. Whereas the discovery of glucocorticoids progressed relatively rapidly, bioassays developed to characterize adrenal cortical extracts proved remarkably insensitive for mineralocorticoid activity despite evidence that unfractionated extracts participated in Na⁺ and K⁺ metabolism. Addition-

ally, controversy emerged over whether biologically important mineralocorticoids existed, with a majority of researchers maintaining that glucocorticoids were the major source of mineralocorticoid activity. For almost 20 yr these obstacles hampered attempts to crystallize what had been termed "electrocortin." Nevertheless, Kuizenga and Cartland (3) (1936–1939) had reported adrenocortical preparations containing "potent mineralocorticoid activity" in the amorphous unfractionated portion. Therefore, investigators—including Nobel Laureate Tadeus Reichstein and his colleagues in Basel, Switzerland; Simpson and Tait in London, United Kingdom; and Kendall and Mason in the United States—remained convinced that electrocortin was a clinically relevant hormone and could be isolated.

By 1948, Deane *et al.* (4), through anatomical and histological examination, determined that electrocortin was secreted from the zona glomerulosa of the adrenal cortex under provocation from a low sodium diet or potassium loading, providing early evidence of regulation. However, it wasn't until 1953, when Simpson and Tait developed a landmark bioassay with high sensitivity for mineralocorticoid activity, that crystallization of 21 mg electrocortin from 500 kg beef adrenal glands was announced (5). Within months, groups in America and Basel confirmed these findings, and by 1954 the structure of aldosterone was reported (6). Highly specific and sensitive bioassays, such as the double isotope derivative assay by Kliman and Peterson (7), introduced a flood of investigations into the regulation and mechanisms of action of aldosterone in subsequent years. It was only 2 yr later that Conn (8) described hyperaldosteronism arising from an adrenal tumor, leading to the identification of the syndrome of hypertension and hypokalemia that bears his name. In 1956, Giroud *et al.* (9) reported production of aldosterone by the zona glomerulosa. Ganong and Mulrow established the limited role ACTH played in aldosterone regulation in 1962 with experiments in hypophysectomized higher animals, determining that although ACTH contributed to aldosterone secretion it was not a major stimulus in humans (10, 11).

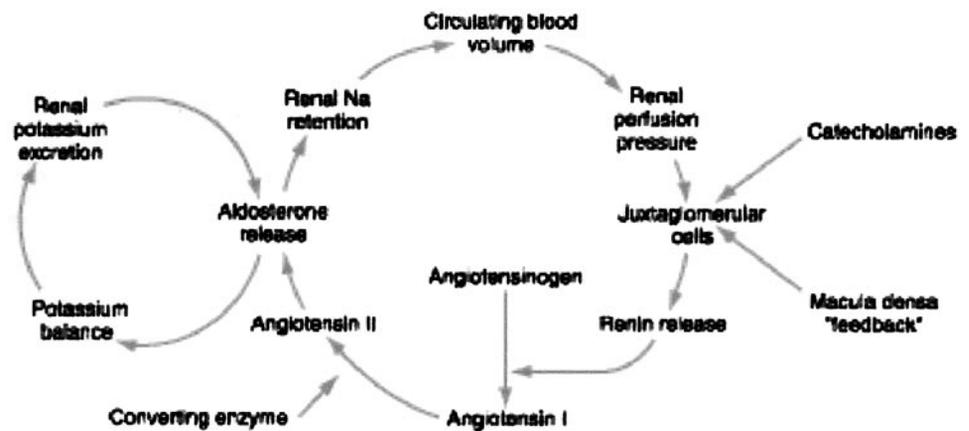
Deane and Mason (12) in 1951 shed light on a major stimulus for electrocortin secretion when they hypothesized that the renin-angiotensin system directly stimulated aldosterone secretion and that this effect could be augmented with dietary sodium restriction. Paige and Helmer (13) a dozen years earlier had isolated a pressor agent, which they initially called angiotonin, also known as hypertensin, and eventually angiotensin. Skeggs *et al.* (14) identified two forms of angiotensin: a decapeptide called angiotensin I and an octapeptide, angiotensin II. In 1958, Gross (15) suggested that

Abbreviations: 11 β -HSD, 11 β -Hydroxysteroid dehydrogenase; ENaC, epithelial Na channel; LVH, left ventricular hypertrophy; PRA, plasma renin activity; RALES, Randomized Aldactone Evaluation Study.

FIG. 1. Contributors of the original manuscript describing the isolation of aldosterone. *Left to right*, Prof. Tadeus Reichstein, Dr. Sylvia A. Simpson, Dr. James F. Tait, Prof. Albert Wettstein, Dr. Robert Neher, and Dr. Joseph von Euw. (Reproduced with permission from *J Steroid Biochem*, vol 3. ©Elsevier Science, 1972.)



FIG. 2. Feedback loops regulating aldosterone secretion. (Reproduced with permission from G. Williams and R. G. Dluhy: *Disorders of the Adrenal Cortex*, McGraw-Hill, New York, 2001.)



the kidney secreted an aldosterone-stimulating factor largely responsible for aldosterone secretion. Three years later, Mulrow and Ganong (16) documented that angiotensin II stimulates aldosterone secretion. The other major stimulus for aldosterone secretion, potassium, was described by Giroud and colleagues (Refs. 17 and 18; Fig. 2). Sodium, calcium, magnesium, and hydrogen along with ACTH were found to play secondary roles in regulating aldosterone secretion.

Since the identification of aldosterone, it had been assumed that it promoted the excretion of potassium and retention of sodium, thereby influencing extracellular volume homeostasis and blood pressure. However, it wasn't until 1958 when Leaf *et al.* (19) developed an isolated monolayer tissue system from toad bladder that a suitable model for the study of mineralocorticoid activity had been established. This enabled classic endocrinological studies of sodium and potassium transport in response to aldosterone and its antagonists.

By the late 1960s, the biosynthetic pathway from cholesterol to pregnenolone to progesterone to 11-deoxycorticosterone to corticosterone and finally 18-hydroxycorticosterone to aldosterone had been established with little

controversy. Although all zones of the adrenal cortex contained most of these enzymes, in humans only the zona glomerulosa was found to demonstrate 18-hydroxycorticosterone activity, thus establishing a pivotal regulatory role for the last step in aldosterone synthesis.

Aldosterone antagonists. Amphenone represents the first agent demonstrated to blunt mineralocorticoid action by inhibiting aldosterone biosynthesis (20). The ability of amphenone to alter urinary sodium excretion enabled study of secondary hyperaldosteronism in congestive heart failure and cirrhosis. Later, 17-spirolactone steroids or spiro lactones were developed to antagonize aldosterone and the activity of other sodium-retaining hormones at the renal distal tubule. In the late 1950s, this led to the development of spironolactone as a potassium-sparing diuretic in the treatment of volume overloaded states and primary hyperaldosteronism.

Genomic mechanisms of aldosterone activity. Classic agonist/antagonist experiments in the toad bladder model in the 1960s revealed that a significant lag (30–90 min) existed between the time of aldosterone administration and alterations in Na/K flux (19, 22). Around this time, endo-

crinology entered the field of molecular biology, revealing the genomic actions of glucocorticoid-mediated, and eventually aldosterone-mediated, induction of RNA with subsequent protein synthesis (23). The delayed response in activity represented binding to cytosolic steroid receptors, translocation to the nucleus, interaction with DNA, and finally genomic transcription and translation of effector proteins.

Clinical significance of aldosteronism. Since its structural identification in 1954, a great deal of information has accumulated about the contribution of aldosterone to several medically important diseases. In 1937, Steigler and Reichstein (24) synthesized desoxycorticosterone acetate, intending it to possess potent glucocorticoid activity. However, it was found to have only very weak glucocorticoid activity but surprisingly caused a syndrome of hypokalemia and hypertension. In 1955, Conn (8) described a clinical syndrome of hypertension with hypokalemia resulting from an autonomous aldosterone-secreting tumor of the adrenal cortex. These pathophysiological observations helped clarify the renin-aldosterone interaction. Two categories of hyperaldosteronism emerged: 1) primary hyperaldosteronism, conditions with suppressed plasma renin activity (PRA), and 2) secondary hyperaldosteronism, those diseases with elevated PRA (25). Therefore, increased aldosterone secretion was determined to contribute to some forms of arterial hypertension, disorders of sodium retention and edema, and syndromes of potassium wasting.

The contribution of aldosterone to hypertension, aside from overt primary hyperaldosteronism, remained obscure during this period although several groups, including Conn's (25), felt that aldosterone activity/sensitivity either directly or indirectly contributed to a substantial portion of essential hypertension. In the late 1950s, increased aldosterone in edematous states was described (26). Rather than a primary event, the hyperaldosteronism was assumed to be secondary. The hyperaldosteronism was postulated to contribute to the sodium avid state of these edematous disorders (e.g. congestive heart failure). At the time, investigators assumed that the maladaptive response of aldosterone in these volume-overloaded states was secondary to the need to maintain intravascular volume. Curiously, edema was not a feature of primary hyperaldosteronism in which patients seem to "escape" the salt-retaining effects of aldosterone. Although several hypotheses have been proposed for the mechanism of escape, it still remains elusive (27) and it is likely that several mechanisms are involved.

Extrarenal effects of aldosterone: early description of cardiovascular remodeling. In 1946, Selye (28) reported on "a state of chronic stress" that developed in rats fed deoxycorticosterone acetate, an intermediate in the biosynthetic pathway of aldosterone with mineralocorticoid activity. This state resulted in an "adaptation syndrome," leading to formation of perivascular granulomas visible in the coronary, renal, and systemic vasculature. A pathological consequence was the development of high blood pressure (28) and histological evidence of myocardial necrosis and fibrosis (29). Other investigators confirmed that administration of glucocorticoids reduced granuloma formation whereas mineralocorticoids

induced inflammation and fibrosis in animal models in a yin/yang relationship (30, 31). Unfortunately, these observations remained essentially forgotten for 40 yr but clearly represent the groundwork on which future investigation of nonepithelial effects of aldosterone were formed.

The middle years: 1970–1990

Regulation of aldosterone secretion. The announcements of a sensitive RIA for plasma aldosterone (32) and a preparation of dispersed zona glomerulosa cells (33) were published in 1970. These two events marked a turning point in aldosterone physiological research, providing the necessary tools to perform detailed investigations of aldosterone biosynthesis and regulation. In the 1960s, theories of aldosterone formation divided biosynthesis into an "early pathway" preceding the production of pregnenolone and a "late pathway" in which corticosterone is converted to aldosterone (34). In the 1970s and 1980s, several inhibitors and stimulants of early-phase products were described, reinforcing the multifactorial complexity of aldosterone regulation; however, no substance had been reported to directly modify the late pathway enzyme, aldosterone synthase. Identified inhibitors of aldosterone biosynthesis included atrial natriuretic peptides, adrenal medullary inhibitor, somatostatin, dopamine, ouabain, androgens, glucocorticoids, and select prostaglandins. Direct simulators included angiotensin II and III, ACTH, α -MSH, prolactin, vasopressin, potassium, hydrogen, ammonium, serotonin, histamine, and select prostaglandins.

Perhaps one of the most intriguing aspects of the regulation of aldosterone biosynthesis are the roles of dietary sodium and, to a lesser extent, dietary potassium. In the early 1970s there was an extensive debate on whether sodium intake had an effect independent of its modulation of angiotensin II levels (30, 35–37). Several investigators as early as 1956 reported that angiotensin II-induced aldosterone secretion was modified by the level of sodium intake (38). A high salt diet blunted the aldosterone response to low-dose angiotensin II infusion as compared with low sodium diet in humans, but not in all experimental animal models. Alterations in aldosterone synthesis and secretion in response to dietary sodium maneuvers were considered to represent a tight link between aldosterone and the renin-angiotensin system activity. Additionally, short- and long-term effects of sodium depletion on aldosterone sensitivity could be replicated by exogenous administration of renin or angiotensin II, whereas in a bilaterally nephrectomized model aldosterone increase was not present. Finally, blockade of renin-angiotensin system activity abolished the aldosterone stimulatory effect of a low sodium diet (39, 40). However, the studies by Tait and Tait and their colleagues (33) and Williams *et al.* (41) clearly documented an independent role for sodium intake both in experimental animals and humans. Their results documented that sodium restriction and potassium loading enhanced the activity of the late pathway (aldosterone synthase) of aldosterone biosynthesis with consequent changes in the structure of the glomerulosa zone of the adrenal cortex and the acute response of aldosterone to angiotensin II and potassium administration. Hollenberg and Williams also documented that sodium intake had a

reciprocal effect on the vascular (particularly renal vascular) response to angiotensin II but not to other pressors (31, 42). They proposed that effect of sodium intake on these two systems was designed to fine tune the organism's response to sodium challenges to more precisely regulate sodium/volume homeostasis. Before these studies, it was generally assumed that the renin-angiotensin-aldosterone system was primarily involved in blood pressure regulation. With this new evidence, it was likely that its primary role was volume regulation. These findings in normal subjects provided the critical underpinnings for subsequent theories in human hypertension.

The discovery of secondary messenger systems removed the former "black box" of signal transduction at the adrenal cortex, thereby expanding the capabilities of cellular biology. The first step in the angiotensin II effect on aldosterone secretion is binding to the type I receptor (AT_1). This is a G protein-coupled receptor that activates protein kinase C generating inositol triphosphate and 1,2 diacylglycerol. These then lead to an increase in calcium release, stimulating aldosterone synthesis (43). In contrast, ACTH activates adenylate cyclase, leading to production of cAMP (44), which in turn, as a secondary messenger, activates intracellular protein kinases (45), thereby leading to a cascade of calcium influx, further modulation of protein kinase activity, and subsequently aldosterone production. An elevation in extracellular potassium opens potential-dependent calcium channels. This increases intracellular calcium concentration and activates cAMP and protein kinases, leading to increased aldosterone synthesis (46, 47). Over the next 15 yr, these studies lead to an extensive evaluation of the interaction of several proteins, ions, and enzymes in the regulation of aldosterone secretion.

Aldosterone in essential hypertension. In the 1960s, several groups maintained that aldosterone played an important role in human hypertension. In the early 1970s, a progressive hypothesis that altered target tissue sensitivity to angiotensin II, aldosterone, renin, and sodium intake together could contribute to the development of human hypertension. Efforts were made to categorize large heterogeneous populations of essential hypertension into homogeneous subsets by comparing hormonal responses to biological stimuli to facilitate investigation of pathophysiological candidates including aldosterone. One of the first substantial subsets identified was low renin hypertension. In this form of hypertension, patients display low PRA despite sodium restriction (48, 49). However, these subjects do not display unusually high aldosterone levels or hypokalemia and suppress aldosterone on high salt diet and are, therefore, distinct from primary hyperaldosteronism. An extensive search for other mineralocorticoids over the next decade identified many potential candidates, but in only a few low renin patients were any of them pathophysiologicaly involved (50).

Based on their earlier work in normal subjects, a second large subset of hypertension was described by Williams and Hollenberg and colleagues (51, 52) in the mid 1980s in which parallel alterations in both adrenal (aldosterone) and renal (renal blood flow) activity in response to angiotensin II and dietary sodium manipulation were described in 25% of the

essential hypertensive population. In these normal/high renin individuals, changes in sodium intake failed to produce the reciprocal changes in adrenal and renal vascular responses to angiotensin II infusion; thus, was born the term "non-modulators." Over the ensuing 15 yr, it has been determined that these individuals have salt-sensitive hypertension (like low renin subjects), are insulin resistant (even if lean), and have a heritable form of hypertension that is associated with polymorphisms in the genes of the renin-angiotensin-aldosterone system. Most intriguingly, their pathophysiological defects are corrected by converting enzyme inhibitors (53–57). Thus, between the low renin and non-modulating hypertensive populations, nearly all of the salt-sensitive subgroups have been identified.

The mineralocorticoid receptor. Historically, adrenal steroids had been classified according to their relative potency as a mineralocorticoid or a glucocorticoid. Similarly, receptors were categorized according to relative affinity. Mineralocorticoid (type I) receptors possessed high affinity for mineralocorticoids and glucocorticoid (type II) receptors possessed high affinity for glucocorticoids. Therefore, early studies using [3 H]-labeled steroid-binding assays demonstrated high affinity for aldosterone and deoxycorticosterone but low affinity for dexamethasone and corticosterone for the mineralocorticoid receptor (58). However, contradictory models developed when attempts were made to explain aldosterone activity at the distal renal tubule in which varying concentrations of mineralocorticoid and glucocorticoid receptors were located and, at times, counterintuitive concentrations of aldosterone or cortisol (59). Clarifying this paradox were experiments performed in 1987 in which adrenalectomized rats were found to have equivalent renal cytosolic *in vitro* binding affinity for aldosterone and corticosterone. However, when these steroids were then injected into the animal *in vivo* significantly higher binding affinity for aldosterone was found in the kidney, parotid, and intestine but not in other tissues (60). The presence of 11β -hydroxysteroid dehydrogenase (11β -HSD) in these tissues conferred high aldosterone selectivity. Enzymatic conversion of cortisol to cortisone by this enzyme disabled the cortisol effect on the type I receptor. This observation explained the phenomenon of apparent mineralocorticoid excess in which deficiency of 11β -HSD lead to a syndrome of cortisol-dependent type I receptor activation with ensuing hypertension and hypokalemia (61). This also explained licorice toxicity resulting in a similar syndrome in which glycyrrhizic acid was a potent inhibitor of 11β -HSD production (62).

Cloning and sequencing techniques introduced in the mid 1980s greatly enhanced efforts to define physiology. The advances clarified how steroid hormones control gene function. By 1987 the mineralocorticoid receptor had been cloned (63) enabling detailed study of regulatory DNA sequences, hormone response elements, and the ability to influence transcription/translation of inducible protein elements. These studies also clarified the molecular mechanisms underlying the earlier *in vitro* studies. Corticosterone and aldosterone bind equally well to the type I glucocorticoid (*i.e.* mineralocorticoid receptor). Thus, the selectivity of the type I receptor

for mineralocorticoids does not occur at the receptor level but at the level of 11β -HSD type II—“the guardian of the gate” (64). In those tissues possessing this enzyme in close proximity to the receptor (e.g. epithelial cells in kidney, bladder, gastrointestinal tract, sweat and saliva glands, smooth muscle, and vascular endothelium), only mineralocorticoids can activate the receptor. In other tissues (e.g. brain and myocytes) that do not possess 11β -HSD type II, glucocorticoids, because of their higher concentration, likely are the principle activators of the mineralocorticoid receptor.

The later years: 1990–2000

Sodium transport at the distal renal tubule. By 1990, substantial research reinforced aldosterone as the primary hormone of Na^+ and K^+ homeostasis, extracellular fluid volume, and blood pressure, yet still unidentified were the aldosterone-induced proteins that bridged aldosterone action at the nucleus and increased sodium transport. In the mid 1990s aldosterone was shown to exert its effect through the epithelial Na channel (ENaC) via latent transcriptional and translational regulation, leading to *de novo* synthesis of ENaC with selective insertion in the apical epithelial membrane effecting enhanced sodium transport (65, 66). Recently, the serum and glucocorticoid-induced kinase, a serine/threonine kinase, was identified as an aldosterone-induced protein that produced upregulation of ENaC (67), thereby completing a link between aldosterone introduction at the cell nucleus to the outcome of Na^+ flux through the ENaC. Traditionally, it has been assumed that the reabsorption of sodium through ENaC modifies the electrochemical gradient for potassium producing an influx into the urine. However, whether this is the only mechanism responsible for potassium excretion is uncertain.

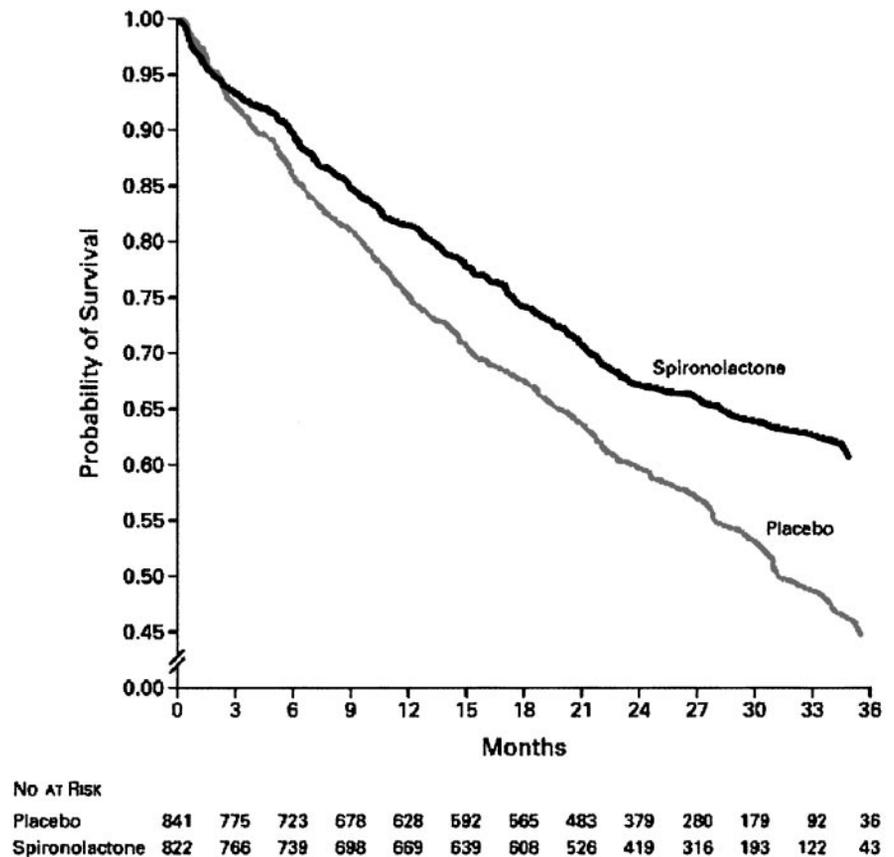
Nonepithelial effects of aldosterone. For most of the past 50 yr the focus of aldosterone action resided in the classical target organ, the kidney. However, as noted previously, Selye and others in the 1950s realized that aldosterone exerted non-epithelial activity particularly relating to induction of inflammatory processes, collagen formation, fibrosis, and necrosis. In addition, steroid hormone biosynthesis had been described in several extra-adrenal tissues including brain, cardiac tissue (68), and blood vessels (69) in which paracrine and autocrine properties are assumed to exist. Studies have revealed the presence of mRNA for aldosterone synthase and aldosterone along with local tissue effects in these tissue beds. In the heart, this local production of aldosterone can be induced by angiotensin II or dietary sodium/potassium manipulations (70). By the early 1990s, interest in this aspect of aldosterone action resurfaced and reports of perivascular fibrosis and cardiac damage appeared in the literature again under various experimental models (71, 72). These nonclassical descriptions of aldosterone activity in nonepithelial target tissues have now been associated with various models of pathology in animals and in humans. Perhaps one of the most intriguing examples of a successful translational research endeavor involved the studies of Weber and Pitt. In the early 1990s, Weber and colleagues (73) studied the effects of aldosterone in cardiac remodeling documenting that chronic aldosterone excess in the presence of salt loading

caused cardiac fibrosis in experimental animals. Additional animal studies demonstrated that uninephrectomized rats fed a high salt diet and aldosterone were protected from development of cardiac fibrosis with the administration of spironolactone (an aldosterone receptor antagonist), thus implicating the mineralocorticoid receptor in cardiac fibrosis. Similar studies in stroke-prone hypertensive rat support the notion that mineralocorticoid blockade reduces vascular injury (74). It should be pointed out that injury was not seen on low salt diet. Further clinical evidence that implicate the likely pathological influence of aldosterone in cardiac disease came from studies of patients with primary hyperaldosteronism. A study comparing severity of left ventricular hypertrophy (LVH), an independent risk factor of increased cardiovascular risk, in patients with primary hyperaldosteronism *vs.* those with essential hypertension found that primary hyperaldosteronism possessed a significantly higher severity of LVH despite controlling for age, duration of hypertension, gender, and severity of hypertension. Napoli *et al.* (75) demonstrated that patients with primary hyperaldosteronism had significantly more impairment in myocardial perfusion as measured by single photon emission computed tomography scan after exercise as compared with matched subjects with essential hypertension. These findings, along with the well documented observation of chronic renin-angiotensin-aldosterone system activation in congestive heart failure, and the finding that blockade of angiotensin was beneficial in this condition led Pitt and colleagues to devise the Randomized Aldactone Evaluation Study (RALES). This multicentered international clinical trial comparing standard therapy in moderate to severe heart failure *vs.* standard therapy plus low-dose spironolactone was terminated early when a 30% reduction in rate of death in the active drug arm was realized (Fig. 3; Ref. 76). Further supporting the role that both cardiac fibrosis and aldosterone effect had in this population was evidence from a substudy of RALES in which markers of collagen synthesis, associated with development of cardiac fibrosis, correlated with disease severity and increased cardiac risk. In the spironolactone-treated subjects, survival benefit correlated with reduced collagen synthesis markers at 6 months (77).

Myocardial fibrosis seems to be a reparative process secondary to inflammatory necrosis (Fig. 4; Refs. 78 and 79). Animal models demonstrate alterations in Na,K-ATPase activity in cardiomyocytes treated with aldosterone lead to necrosis, recruitment and activation of macrophages, secretion of growth factors including TGF- β , and subsequent reparative fibrosis and dysfunction. An important component of these events is the presence of dietary sodium. In low sodium environments, the negative effects of aldosterone administration were markedly blunted (80). Furthermore, although small increases in potassium may contribute to the beneficial effects of mineralocorticoid receptor blockade, animal studies have documented a protective effect of blockade independent of an increased potassium (80).

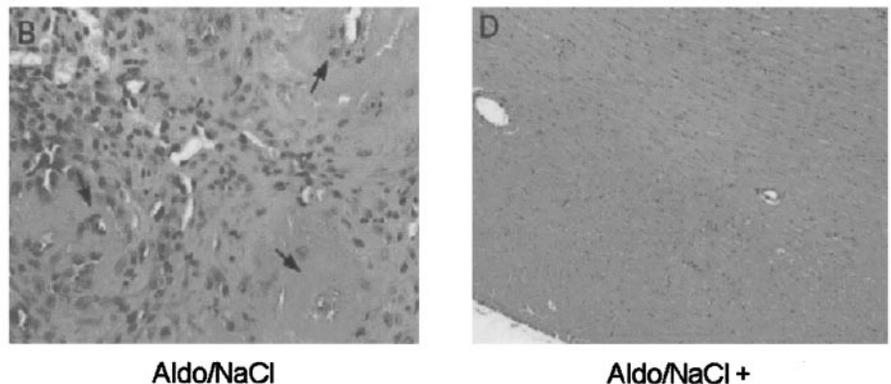
Nonclassical aldosterone action: rapid nongenomic effects of aldosterone. Earlier studies demonstrated a lag between times of administration of aldosterone to increased cellular activity (~ 30 – 90 min). Subsequent research focused on revealing the

FIG. 3. Survival curves among patients treated with placebo or spironolactone. Risk of death was 30% lower in the spironolactone-treated group in the RALES trial ($P < 0.001$). [Reproduced with permission from B. Pitt *et al.*: *N Engl J Med* 341:709, 1999 (76).]



Myocardial Histopathology in Aldosterone/NaCl-treated Hypertensive Rats

FIG. 4. Photomicrograph of myocardium in rats treated with aldosterone and salt with and without eplerenone. Note the presence of vasculopathy including perivascular inflammatory cell infiltrate in rats treated with aldosterone and the absence of these findings in rats treated with aldosterone and eplerenone [Reproduced with permission from R. Rocha *et al.*: *Am J Physiol Heart Circ Physiol* 283: H1802–H1810, 2002 (78).]



cytosolic steroid receptors to which aldosterone binds. These receptors translocate to the nucleus and function as transcription factors through interaction with DNA regulatory elements (81, 82). As mentioned earlier, two distinct nuclear receptors were characterized: the mineralocorticoid and glucocorticoid receptors. The ENaC, K^+ channels and Na^+/K^+ -ATPase are the final effector elements of this genomic interaction. In general, this is described as classical genomic effect of aldosterone. However, research over the last 10 yr has led to the discovery of rapid, nongenomic or nonclassical

effects of aldosterone. This has led to investigation of aldosterone action in tissues other than the kidney. In 1992, Wehling *et al.* (83) identified rapid (nongenomic) effects of aldosterone action in smooth muscle (83). Reports of rapid nongenomic effects of aldosterone have been described in skeletal muscle (84), colonic epithelial cells (85), and myocardial cells (86). These nongenomic effects have been linked to development of increased systemic vascular resistance (87) and might, therefore, participate in human hypertension and cardiovascular disease. However, it is uncertain whether

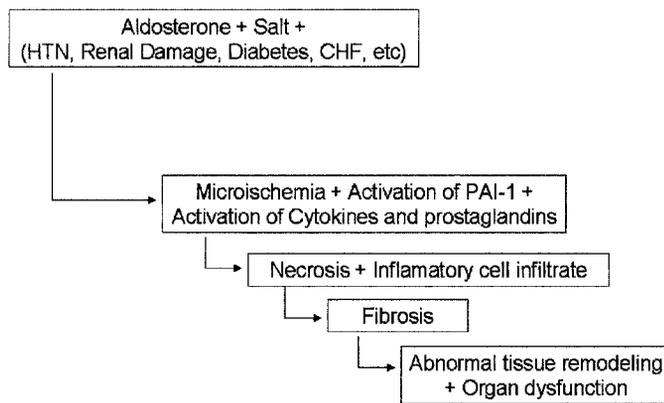
Propose Mechanism for Aldosterone Induced Cardiovascular Injury

FIG. 5. Suggested multistep process leading to organ dysfunction in individuals or animals in the presence of aldosterone and salt.

these effects are secondary to activation of the classical mineralocorticoid receptor or not.

The 21st century

It is only in the last 10 years that a flurry of academic interest has encircled the potential influence of aldosterone in a wide variety of diseases. Much of this excitement derives from the recently discovered nonclassical pathways of aldosterone action, the presence of extra-adrenal aldosterone production, and the rediscovery of mineralocorticoids as proinflammatory and profibrotic molecules (Fig. 5). Essentially, any tissue expressing the mineralocorticoid receptor is now a target of investigation. This also has led to a renewed interest in the relative cardiovascular protective roles of blockade of angiotensin II formation or effect *vs.* blockade of aldosterone formation when angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers are administered. Two recently completed studies [The Eplerenone, Enalapril, Eplerenone and Enalapril (4E) studies] have addressed this question. Both studies had a similar design. In one, hypertensive subjects with LVH (4E-LVH) were enrolled (88). In the second, hypertensive subjects with diabetes and proteinuria (4E-DM) were studied (89). Subjects were randomized into maximum tolerated doses of enalapril, eplerenone (a new selective aldosterone antagonist with virtually no effects on sex hormones), or the combination. The results on these cardiovascular target organs were nearly the same: blocking the mineralocorticoid receptor was as effective, if not more effective, in reducing LVH or proteinuria as blocking the formation of angiotensin II. Intriguingly, the combination produced a substantially greater reduction in target organ damage than either therapy alone suggesting both angiotensin II and aldosterone are involved in mediating damage. These studies suggest that aldosterone may be involved to a greater extent in human hypertension than what had been previously assumed. In addition, although still controversial, as many as 10% of the hypertensive population may have excess aldosterone production (*i.e.* primary aldosteronism; Ref. 90).

A recently completed mortality trial provides additional support for the hypothesis that aldosterone in the presence

of salt is a cardiovascular risk hormone. In EPHEsus (Eplerenone Post-AMI Heart Failure Efficacy and Survival Study), patients who developed congestive heart failure acutely following a myocardial infarction were randomized to standard therapy with or without low-dose eplerenone. As with the RALES trial, there was a striking mortality reduction in those patients treated with eplerenone (91).

Conclusions

Fifty years of fruitful and exciting experimentation and discovery have elapsed since the isolation and characterization of aldosterone by Simpson and Tait. It seems ironic that considering the role of aldosterone teleologically as a relatively recent and advanced adaptive mechanism to assure sodium and volume homeostasis that it is implicated in maladaptive consequences in the setting of volume overload states such as hypertension, nephrosis, heart failure, and cirrhosis. The mechanisms by which this occurs are still being unraveled. Certainly, further study is needed to investigate what role functional polymorphisms might play in possible predisposition to developing this maladaptive response and critically how dietary sodium converts the effect of aldosterone from physiological to pathological. Regardless, the historical review of the discovery of aldosterone emphasizes the importance of recognizing early contributions to medical research in the setting of technological advances.

Acknowledgments

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