The Local Cardiac Renin–Angiotensin Aldosterone System

Second Edition

Edward D. Frohlich · Richard N. Re
Editors

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Springer

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Preface

With great pleasure we present our Third International Workshop on The Local Cardiac Renin–Angiotensin–Aldosterone System (RAAS), held at the Ochsner Clinic Foundation in November 2008. When this meeting was first organized in 2002, we were pleased that all who were invited to participate in our first workshop attended our program; and what is most satisfying, all of these participants returned for the second and this, the third workshop. In addition, we are delighted that a number of additional workers in this growing field of investigation joined us for this third program.

Over the years since our first workshop, there is little doubt as to the existence of a local RAAS in the heart. Indeed, there is substantial evidence as to the existence of other local systems in brain, vessels, adrenal, and, as presented in the current workshop, the kidneys. What is even more stimulating intellectually, through duplication of local RAAS systems a remarkable yin-yang biological balance is provided by nature. Thus, in the current third workshop considerable evidence has accumulated over the years for complicated local systems that serve to provide exquisite and unique local control of local organ functions.

This notion that local renin–angiotensin systems (RAS) operate in a variety of tissues has gained considerable importance over recent years. Although not all components of the RAAS need be synthesized in a particular tissue, local regulation of the production of angiotensin II and other angiotensin-related peptides can, nonetheless, reside at the tissue level by virtue of differential uptake of some system components and regulated synthesis of others. Moreover, the recognition that renin receptors exist in target tissues expands the possible physiological implication of local RASs by defining a new mechanism through which (pro)renin can alter tissue biology. In addition, the angiotensin (1-7)/ACE2 arm of the RAS is proving to offer important new insights into the workings of these systems in health and disease. Similarly, the local actions of aldosterone—and the possibility of the extra-adrenal synthesis of this hormone—further expand the role of the local RASs. The AT-2 angiotensin receptor is also proving to be important in previously unappreciated ways in mediating the tissue effects of the RASs. So too, new insights into the workings of the AT-1 receptor offer the prospect of better understanding the local regulation of angiotensin action. Finally, a large and growing body of evidence has recently been developed to indicate the intracellular or intracrine action of angiotensin II

and other RAS components, thereby extending the notion of the local RASs to the intracellular milieu.

This monograph is dedicated to disseminating new findings on all these levels with a focus on the local RAASs of the cardiovascular system and kidney. It is derived from the papers presented and discussed at the Third International Workshop on The Local Cardiac Renin Angiotensin Aldosterone System held November 12–14, 2008, on the campus of the Ochsner Clinic Foundation in New Orleans, Louisiana, and builds on the reports of the Ochsner Workshop of 2002 and 2004. The editors and organizers once again are extremely pleased with the willingness of outstanding investigators in the area of RAAS biology to participate both in the symposium and in the production of this monograph. We thank them for their willingness to share their latest research findings. As a result of their efforts, the editors are confident the resulting monograph will prove to be of considerable value to anyone interested in this emerging and important field of inquiry.

Finally, we wish to extend appreciation to the AstraZeneca and Forest Pharmaceutical firms, which provided support to this workshop. There were no statements of commercial interests in any of the participants' contributions before, during, or after the workshop. And, finally we wish to express the personal appreciation of the participants for the administrative support of the workshop by our staff, Mrs. Lillian Buffa and Ms. Caramia Fairchild.

New Orleans, Louisiana
20 March 2009

Edward D. Frohlich
Richard N. Re

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Chapter 1
Are Local Renin–Angiotensin Systems the Focal Points for Understanding Salt Sensitivity in Hypertension?

Edward D. Frohlich

Abstract Salt has had a prominent role in the history of man. Initially involving social, economic, and political aspects of human endeavor, in more recent decades salt has become extremely important in its role in the pathogenesis of cardiovascular and renal diseases. The magnitude of this relationship is of tremendous significance, affecting the health of billions of people throughout the world. Our laboratory studies in the adult spontaneously hypertensive rat and in its normotensive control Wistar Kyoto rat over the past 30 to 40 years have clearly demonstrated that in addition to elevating arterial pressure slightly, but significantly, long term salt loading produced severe structural and functional derangements of the vital organs. These salt induced changes have resulted in severe fibrosis (with deposition of hydroxyproline, type 1collagen), ischemia of both ventricles (the hypertrophied left as well as the non-hypertrophied right), and impaired diastolic ventricular function in the presence of preserved systolic function. The aorta demonstrated severe fibrosis and impaired distensibility and pulse wave velocity. Furthermore, the kidneys demonstrated severe changes of nephrosclerosis manifested by marked ischemia, fibrosis, small cell infiltration, glomerular sclerosis, increased total arteriolar resistance associated with afferent and efferent glomerular resistances with increased glomerular hydrostatic pressure, and marked proteinuria. The changes are typical of diastolic functional impairment of the heart and end-stage renal disease in patients with end-stage renal disease that were dramatically prevented and/or reversed by either of two angiotensin II (type 1) receptor blocking agents. These salt induced cardiac, vascular and renal structural and functional findings are strikingly similar to the target organ involvements in patients with essential hypertension associated with suppression of the endocrine rennin–angiotensin system mediated through the juxtaglomerular apparatus, We therefore suggest that these disastrous effects of salt loading are mediated through local cardiac, vascular, and renal angiotensin systems in these organs. They are dramatically supported by a large recent multicenter

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clinical trial involving prehypertensive patients who were maintained on their usual salt loaded diets and were compared with similar patients who received a salt restricted diet. Further studies are in progress to elaborate this attractive and novel mechanism of action.

Salt has had a prominent role in the history of man. Initially involving social, economic, and political aspects of human endeavor, in more recent decades, salt has become extremely important in its role in the pathogenesis of several diseases. This relatively more recent health concern relates to the role of salt in a multiplicity of cardiovascular, renal, endocrine, and neurological diseases. The magnitude of this relationship is of tremendous significance and affects the well-being of billions of people throughout the world although the underlying disease mechanisms are inadequately understood[1].

The association of salt and disease was initially based upon astute clinical observations of patients with hypertension and cardiovascular disease[2]. This relationship was subsequently supported by many epidemiological studies of large population groups which presented substantial evidence demonstrating that the greater the dietary salt intake in less developed as well as more acculturated or industrialized societies, the greater the prevalence of hypertension[3–5]. However, this relationship has been more difficult to show in the everyday clinical practice of medicine in which only a minority of patients (perhaps a third or less) with essential hypertension demonstrate this close relationship. Thus, a disturbing conundrum exists which heretofore has been unexplained[6]. In part, this may be related to two underlying factors: first, hypertensive disease has been defined epidemiologically by blood pressure measurements (albeit carefully obtained) and, second, by the general definition of "hypertension" that is based on the term "salt sensitivity" of blood pressure[7]. This latter term relies exclusively upon the response of arterial pressure to salt loading, a situation that depends upon rather rigidly defined short-term clinical procedures[7]. On the other hand, an alternative means for defining salt sensitivity could also be demonstrated by a significant reduction of arterial pressure and its consequences with sodium or salt withdrawal; but this means also has not ideally been tested systematically[8].

Thus, this situation continues to exist; but it can be approached more appropriately by re-evaluation of the definition of "hypertension" that considers hypertension as not simply the significant elevation of arterial pressure. Hypertension also depends upon the precise demonstration of the structural and functional alterations of the target organ of disease (i.e., heart, aorta, vessels, kidney, and brain) as well with the elevated arterial pressure (of course, associated with evidence of chronic salt overload)[6,9]. Indeed, appreciation of this obvious necessity has been the focus of our experimental investigative activities dealing with salt excess in hypertension over the past 40 years[10–16]. To this end, we have restricted our experimental efforts to studies of the spontaneously hypertensive rat (SHR), a strain which demonstrates naturally developing hypertension without the necessity of other experimental interventions such as ablation of renal mass, other dietary manipulations, or exogenously administered drugs such as steroids[17].

Experimental Investigations

The results of our studies have clearly demonstrated that salt excess, no matter whether by 4, 6, or 8% salt loading, consistently and significantly elevates arterial pressure (if not excessively) in the SHR. Furthermore, these responses have consistently and impressively been associated with cardiac, vascular, and renal pathophysiological changes that are identical to those changes which occur in the patient with essential hypertension (e.g., impaired arterial distensibility, left ventricular hypertrophy with primarily diastolic ventricular dysfunction). In the younger adult SHR, 25% developed systolic dysfunction with cardiac failure, but in all of the older adult SHRs and the remaining 75% of the younger adults developed diastolic dysfunction with preserved systolic function was demonstrated and was also associated with impaired aortic distensibility and nephrosclerosis with end-stage renal disease[15,16]. In addition to the latter changes in the kidney, our renal micropuncture studies (after prolonged salt loading) demonstrated renal arterial resistance increase, ischemia, afferent and efferent glomerular arteriolar constriction, increased glomerular hydrostatic pressure, severe proteinuria, increased serum creatinine and uric acid concentrations, and end-stage renal disease[16].

Clinical Investigations

In addition to the many epidemiological studies demonstrating a highly significant association between dietary intake of salt (i.e., sodium) and those clinical reports demonstrating evidence of salt sensitivity in patients, there have been a number of clinical reports that have demonstrated adverse clinical effects of salt loading. These studies included the effects of salt on arterial pulse pressure, distensibility, and pulse wave velocity[18,19]; ventricular relaxation[20]; renal function and proteinuria[21,22]; and other target organ damage in normotensive and prehypertensive individuals[23] as well as in patients with essential hypertension.

Most pertinent to this discussion was the first multicenter clinical epidemiological study, which only recently demonstrated that dietary restriction of sodium resulted in a significant reduction in cardiovascular morbidity and mortality. This study, conducted in two parts, the Trial of Hypertension Prevention (TOPH) I and II, involved 2,382 participants with "prehypertension" receiving either a sodium-restricted diet or a control normal diet which was not restricted in its sodium content[23]. The clinical outcomes of this study included stroke, myocardial infarction, coronary artery bypass grafting, percutaneous transluminal angioplasty, and cardiovascular death. Patients who had bypass grafts and angioplasty prior to the initiation of the study were excluded[23]. The findings convincingly demonstrated that the risk of cardiovascular events was about 35% lower in those prehypertensive individuals who received the sodium-restricted diet. Earlier prospective evidence supported the earlier epidemiological reports in which only a single determination of urinary sodium excretion was used to demonstrate diminished risk of stroke and coronary heart disease[24,25].

Hypothesis

A reasonable question therefore follows as to an explanation for our findings which demonstrated that salt loading produced an elevation of arterial pressure associated with pathophysiological changes that were identical to those seen in patients with essential hypertension or those "prehypertensive" individuals who received their unrestricted salt-excess diets[14–16,23]. These controlled experimental studies demonstrated changes including impaired large arterial distensibility, diastolic ventricular dysfunction with preserved systolic function, and nephrosclerosis with end-stage renal disease, which are the most common end-points of long-standing essential hypertensive disease[26,27]. Moreover, each of these reproduced experimental observations has been prevented by the co-administration of angiotensin II type I receptor blocking agents even though arterial pressure was not significantly reduced by this treatment[15,16,22]. The most plausible explanation for these findings, to our way of thinking, is that the therapeutic agents used were able to suppress the action of angiotensin II on the target organs of hypertensive cardiovascular and renal disease and, consequently, the elaboration of the structural and functional derangements of hypertension[6,15–17,26,27].

However, classic salt loading is well known to suppress the release of renin from the juxtaglomerular apparatus of the kidney and the subsequent decrease in the synthesis of angiotensin II. Our experimental findings showed that salt loading only slightly, but significantly, raised arterial pressure even though it also produced severely deranged aortic, cardiovascular, and renal diseases, which were markedly prevented individually by two different angiotensin II receptor blocking drugs in separate studies[15,16,22]. We suggest that these therapeutic agents did, in fact, act on angiotensin II type I receptors in these target organs but not by interfering with the classic renal endocrine action of renin. Thus, we hypothesize that the prolonged salt loading stimulated local renin–angiotensin–aldosterone systems in the aorta and smaller arteries; heart, and kidneys. There are much compelling recent data that support the notion that there are operable local renin-angiotensin II type 1 systems, which have been demonstrated in the target organs of hypertension studied in our studies[28–32]. Much work is necessary to demonstrate that these local systems were stimulated experimentally by salt loading, that these systems can be inhibited by angiotensin II receptor blocking drugs, and that hypertensive cardiovascular and renal diseases can similarly be prevented, inhibited, or reversed by these drugs in patients with prehypertension or with established essential hypertension.

There are several biological mechanisms which have been postulated that may explain the above findings. Our hypothesis suggests that local renin-angiotensin systems existing in the heart, arteries, kidney, and other organs are stimulated to initiate mitogenesis, collagen synthesis and fibrosis, apoptosis, and other possible pathological events[6,9]. Among the other biological actions that have been reported are salt-related mitogenesis of the cardiomyocytes; exaggerated accumulation of fibrillar collagen within the extracellular ventricular matrix and surrounding arterioles within the ventricle that are, in part, independent of the hemodynamic load; modulation of the hemodynamic response to norepinephrine, implying overactive

adrenergic function in response to sodium excess; and sodium ion facilitation of a possible role of certain growth-promoting hormones and factors[33]. However, none of these mechanisms have been prevented by an angiotensin II type 1 receptor antagonist.

Hopefully, our data and those of others will stimulate further studies that will provide additional evidence demonstrating that the mechanisms underlying the actions and events which involve the local renin-angiotensin systems in promoting the disastrous adverse clinical outcomes result from dietary sodium excess.

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Chapter 2
Newer Insights into the Biochemical Physiology of the Renin–Angiotensin System: Role of Angiotensin-(1-7), Angiotensin Converting Enzyme 2, and Angiotensin-(1-12)

Carlos M. Ferrario, Jewell A. Jessup, and Jasmina Varagic

Abstract Knowledge of the mechanisms by which the rennin–angiotensin system contributes to cardiovascular pathology continues to advance at a rapid pace as newer methods and therapies uncover the nature of this complex system and its fundamental role in the regulation of blood pressure and tissue function. The characterization of the biochemical pathways and functions mediated by angiotensin-(1-7) [Ang-(1-7)], angiotensin converting enzyme 2 (ACE2), and the mas receptor has revealed a vasodepressor and antiproliferative axis that within the rennin–angiotensin system opposes the biological actions of angiotensin II (Ang II). In addition, new research expands on this knowledge by demonstrating additional mechanisms for the formation of Ang II and Ang-(1-7) through the existence of an alternate form of the angiotensinogen substrate [angiotensin-(1-12)] which generates Ang II and even Ang-(1-7) through a non-renin dependent action. Altogether, this research paves the way for a better understanding of the intracellular mechanisms involved in the synthesis of angiotensin peptides and its consequences in terms of cell function in both physiology and pathology.

Introduction

Knowledge of the mechanisms contributing to the pathogenesis of cardiovascular disease is today at a crossroad, possibly one of its most important stages, due to rapid advances in genetics, cellular signaling mechanisms, and the addition of new therapies. Concepts, often heavily weighted by a reductionist approach to accepting the multi-faceted nature of the mechanisms contributing to organ changes in the evolution of chronic disease processes, have been confronted by new discoveries

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that do not match previous tenets[1,2]. Advances in molecular biology are bringing to the physician new and sometimes bewildering views, which he has to learn and judge in relation to clinical facts and to pressures derived from the new problems generated by advancing technology, earlier diagnosis, and longer survival. The evolution of knowledge on the contribution that the renin–angiotensin system has in the regulation of tissue perfusion in both health and disease is a good example. The past decade brought to the forefront the complexity of the renin-angiotensin system, which is more elaborate than originally accepted. The basic research knowledge of the role of angiotensin II (Ang II) in hypertension, vascular disease, and lipid and carbohydrate metabolism does not necessarily match with the outcomes of clinical trials testing the system's contribution by way of using angiotensin converting enzyme (ACE) inhibitors[1,2], Ang II receptor blockers (ARBs)[3], and now the new class of direct renin inhibitors[4–8]. It will be inappropriate to assume that this relative gap between the lessons that are learnt from testing the effects of these agents in the clinical setting and the information gained from meticulous studies of the renin-angiotensin system in animal models and cell systems suggests that one or the other has gone astray. What we need to remember is that homeostasis, in both health and disease processes, depends on the interplay of multiple regulatory mechanisms, which in the normal state act in coordination while they may become discordant in disease states.

In this chapter, we will address these issues from a viewpoint that for one of us (CMF) originates from perspectives gained from his association with Dr. Irvine H Page and from the research we conducted since the first demonstration of the biological actions of angiotensin-(1-7). Throughout this time, the slow process of unraveling pieces of this puzzle provides a more cogent understanding of the harmonious and dis-harmonious ways by which the renin-angiotensin system works to regulate normal blood pressure and its contribution to the expression of the disease, we call, essential hypertension.

A Revolving Story

Although a discussion of the biochemical pathways accounting for the formation of angiotensin peptides should begin with a description of the role of renin in the formation of angiotensin I (Ang I), for our objective we will begin with the discovery and analysis of the functions of the heptapeptide angiotensin-(1-7) [Ang-(1-7)], since its characterization became the stepping stone for a new understanding of the renin-angiotensin system. At the time of the first report of a biological effect of Ang-(1-7)[9], investigators were adamantly focused on finding a receptor for the actions of Ang II. Work on Ang II analogs and Ang II peptide antagonists suggested that the Pro^7–Phe^8 bond of the Ang II molecule was an essential requisite for binding to the as yet to be identified receptor[10–12]. Therefore, our first report that Ang-(1-7), having a truncated C-terminus, showed biological activity did not meet with any enthusiasm. Over the ensuing years, and as reviewed elsewhere, our laboratory continued to unravel the participation of Ang-(1-7) in the regulation of