Session VII. Research and Public Health Directions

Salt and Hypertension — Future Directions

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The comments that follow reflect the personal views of this author regarding some useful directions of research in the field of salt and hypertension. From a physiological perspective, salt sensitivity is defined, and the merits and limitations of certain animal models of hypertension used to study this issue are discussed. The need to more clearly define the mechanisms that detect sodium intake and control the renal excretion of sodium is discussed. Additionally, the need to better understand the relation between sodium homeostasis, volume regulation, and the consequences of dysfunction in these regulatory systems on the arterial vasculature and interstitial matrix is emphasized. The necessity for the application of new tools and approaches in a number of investigative areas is discussed. Finally, the necessity of equally imaginative whole animal and cell/molecular research and efforts to merge and integrate the data obtained at the cellular level with that of intact systems is emphasized. (Hypertension 1991;17[suppl I]:I-205–I-210)

Although considerable progress related to issues of salt and hypertension has been made over the past decade, this workshop manifests evidence of many areas that remain unclarified. The following comments address some of the problems that remain unresolved and suggest some of the future research directions that might be productively pursued.

What Is Salt Sensitivity?

By the term salt, we are generally referring to sodium chloride, or table salt. Evidence indicates that the anion chloride must accompany sodium to be an important determinant of arterial pressure. However, as seen in this workshop, the term “salt sensitivity” has not been clearly defined, which has made for difficult scientific dialogue. To some investigators, salt sensitivity implies an immediate rise of arterial pressure as salt intake is increased. Yet it is well-known that sodium balance at any level of arterial pressure requires 3–4 days to achieve, so immediate responses of pressure seen with acute saline loading or volume reduction induced by a diuretic largely reflects the capabilities of reflex mechanisms to respond to blood volume changes and are of negligible value in predicting long-term salt sensitivity. Other investigators view salt sensitivity more as a gradual rise of pressure to the diseased state of hypertension in response to a lifetime of daily exposure to a high salt diet.

Acceptable criteria clearly need to be established to determine whether the arterial pressure and systemic vasculature are significantly influenced by alterations of sodium chloride intake. This alteration of salt intake or excretion for a few hours is too short and for a lifetime too long. The ultimate goal would establish criteria or markers that could predict whether prolonged high salt intake would lead to sustained hypertension. This objective should represent an important area of future research. However, at this time clinical research in human subjects would benefit by standardization of short-term tests to identify subjects who exhibit elevation of arterial pressure in response to increased salt intake. It would be meaningful to test for changes of mean arterial pressure after a stable 1-week control period, in which the subjects are maintained for a period of 1 week on a standardized reduced salt intake of 70–80 meq NaCl/day followed by a 1-week period at an increased level of 200–250 meq NaCl/day.

During formulation of a definition of salt sensitivity, it is important to recognize that, from a physiologist’s point of view, we are all salt-sensitive when the level of salt and water intake can no longer be excreted at a normal level of renal perfusion pressure. Some individuals clearly have less capacity to excrete increases of salt intake and can only do so with elevations of the renal perfusion pressure. The mechanisms that regulate body sodium content are for this reason probably the most important long-term determinants of arterial blood pressure. Reduction of renal excretory ability in time leads to an elevation of arterial pressure, which evidence has shown is necessary to restore sodium balance to a normal level. The factors we have searched for in
our many experimental models of hypertension are those that can cause reduced ability to excrete sodium chloride, consequently raising arterial pressure. The determinants of salt-sensitive forms of clinical hypertension are multifactorial. The hallmark of these experimental forms of hypertension is the reduced ability of the kidneys to excrete adequate amounts of sodium at a normal level of renal perfusion pressure. This dysfunction can be a result of intrinsic alterations of renal function or secondary to alterations of the many extrinsic neural or hormonal controllers of sodium chloride excretion. Such alterations may be genetically determined tubular transport defects, a result of excess sodium-retaining hormones (renin-angiotensin-aldosterone and deoxycorticosterone acetate), or a result of excess renal sympathetic nerve activity driven by the central nervous system. Renal damage and reduced excretory ability can accompany aging in subjects with essential hypertension and can result from infections, autoimmune diseases, and other secondary causes.

Obviously, much remains unclear that concerns the mechanisms whereby changes of salt intake influence arterial pressure. Within a heterogeneous population of normotensive humans or rats, arterial pressure that is sometimes inversely related to salt intake has been observed. Although one suggestion is that this outcome results from suppression of renal renin, many other mechanisms that contribute to sodium and water homeostasis are also probably involved in these responses and require further exploration.

Because of the pivotal role of the kidney in sodium homeostasis, greater attention has, surprisingly, not been devoted to the development of more meaningful ways to assess its function. Future research should focus greater effort toward a noninvasive assessment of the ability of the kidneys to excrete sodium and water in the absence of a counterregulatory reflex and hormonal effects that obscure the relation between arterial pressure and urine excretion. This information could provide a meaningful and direct marker for the potential development of hypertension from excess salt intake.

Animal Models of Salt-Sensitive Hypertension

Obviously, the mechanisms that confer salt sensitivity on various experimental models of hypertension are poorly understood. Physiological studies have tended to be descriptive and qualitative in nature. Despite the progress that has been made in the identification of various mechanisms that can influence sodium and water excretion, a poor understanding of the relative importance of many of these mechanisms remains. Until recently, few investigators have focused on the way in which salt itself could increase blood pressure. Excess salt was offered because it enhanced the rise of pressure in many models of hypertension. Although the effects of hypertension were effectively studied with these models, the mechanisms whereby salt influenced blood pressure were often not clearly addressed. However, from these studies it became apparent that some models of hypertension were more affected by salt than others.

Some experimental models of salt-sensitive hypertension offer a better chance of defining basic mechanisms than others. If they have been appropriately characterized and inbred (such as the Rapp rat strain), the genetic models of salt-sensitive hypertension are of great importance to those interested in defining the genetic links to salt sensitivity, but because these models have polygenic defects, determination of how the excess salt intake may in itself inflict damage to normal animals is difficult. Conversely, methods used to render animals sensitive to the influence of salt intake must be carefully considered. For example, excess administration of deoxycorticosterone and salt is of limited use because deoxycorticosterone alone results in a variety of metabolic, endocrine, and cardiovascular changes that greatly complicate our ability to understand the influence of salt per se. Other drug-induced models are similarly limited. The classic one-kidney, one clip model of Goldblatt hypertension in rats is one of the better-characterized experimental models of hypertension, showing normal levels of plasma renin activity after the rise of arterial pressure, and is a salt-resistant model of hypertension. In contrast, the two-kidney, one clip rat model is salt-sensitive and therefore a useful model for such studies.

Future studies aimed at defining the mechanisms whereby excess salt can raise blood pressure should use better-defined and simpler models in which more of the variables can be controlled. One such model in dogs and rats is the sensitivity to salt that is conferred by physical reduction of renal mass from 65% to 80% of normal size. These animals remain normotensive with nearly normal endocrine profiles until placed on a high salt diet. The only requirement for hypertension after removal of renal mass is excess salt, so that the complications of other drug and hormone effects are minimized. One could then study the consequence of sodium retention in its purest form. Another very useful and highly reproducible model is produced by increases of salt intake in dogs that receive a fixed intravenous infusion of angiotensin II administered at a rate that maintains nearly normal plasma levels during high salt intake.

Detectors of Body Sodium and Effector Mechanisms

Many unresolved questions remain on how the body detects changes in sodium intake. Because the organism cannot detect total body sodium content, theories have been based on detectors of sodium concentration or the detection of changes in body fluid volumes that accompany sodium intake changes.

Stretch Receptor Mechanisms

Despite the evidence that cardiopulmonary receptors can influence many neural and hormonal factors that affect renal excretory mechanisms, the quantita-
tive contributions of these various pathways are not well understood. Many theoretical limitations are imposed by stretch-receptor control systems. These systems adapt quickly and require a sustained error signal for operation. Increasing evidence reveals that these mechanisms may not be major long-term controllers of sodium and water balance. A better understanding of the detection of sodium intake changes is crucial to our knowledge of salt and hypertension and is an important area for future research. Future studies need to quantify the role of these control systems in normal and hypertensive states.

Central Nervous System Salt Detector

When cerebrospinal fluid (CSF) sodium concentration increases, arterial pressure increases as a result of increased sympathetic nerve activity and renin and vasopressin secretion. Yet neither plasma nor CSF sodium concentration appear to change in normal animals or humans over wide ranges of salt intake if drinking water is available. Secondary changes associated with hypertension, which could alter these relations, require exploration in addition to hereditary structural and functional differences. For example, both salt-sensitive hypertensive humans and Dahl salt-sensitive rats reportedly exhibit a rise in CSF sodium concentration when placed on a high salt diet. Normotensive and hypertensive humans, dogs, and rats that are not salt-sensitive exhibit no measurable changes in CSF sodium concentration over wide ranges of sodium diets.

The central nervous system or systemic detectors of sodium concentration may be reset to a different operating level, with consequent alterations in sodium homeostasis and arterial pressure. This process could provide a mechanism whereby the concentration of extracellular sodium and afferent stretch detector activity could be normal, with enhanced central responsiveness to this information.

Control of Sodium Excretion

A variety of neuroendocrine and physical factors are believed to participate in sodium excretion. These factors include the renal sympathetic nerves, the renin-angiotensin-aldosterone system, vasopressin, renal autocoitids such as prostaglandins and kinins, atrial natriuretic factor, and the physical forces of renal perfusion pressure and plasma oncotic forces. Much knowledge has been gained about the cellular mechanisms of actions of most of these effector mechanisms.

Yet there remains a deficient understanding of the hierarchy of many of these controllers and their relevance to sodium homeostasis and hypertension. Recently, dogs have shown efficient excretion of isotonic saline loads in the absence of changes in renal nerve activity or any of the currently identified circulating hormones. Other major-identified factors or underestimated effects of known factors seem to significantly participate in sodium excretion. Future research needs to identify and quantify the contributing mechanisms. Although hopefully the discovery of atrial natriuretic factor might have been the significant influence, it also might not have been. At this time, insufficient evidence exists that it represents an important short-term regulator of sodium excretion, but future studies will require exploration of the long-term physiological and pathophysiological roles of atrial natriuretic factor.

Physical factors also require careful reexamination because they can detect excess salt and water and directly initiate changes of renal excretion. The acute pressure-diuresis response has shown much more sensitivity than previously thought, and reduced sensitivity of these responses is evident in experimental forms of salt-sensitive hypertension. Future studies require direction toward a better understanding of the mechanism whereby these relations are modified in salt-sensitive forms of hypertension. Recent application of laser-Doppler flowmeter techniques to the measurement of renal papillary blood flow offers a powerful new tool to explore such changes. The application of these techniques indicates that papillary blood flow is not sufficiently autoregulated and may play a pivotal role in the pressure-natriuresis mechanism.

Another potentially important physical factor that has not received sufficient attention is the role of changes in colloid osmotic pressure (COP), which occur with acute increases of salt and water intake. Earlier studies that evaluated COP effects on urine excretion were performed before the discovery of many factors now known to influence sodium excretion and were therefore not well controlled. Because changes of COP can substantially alter renal excretion, these relations need to be explored in salt-sensitive forms of hypertension.

Relation of Volume and Arterial Pressure

Because sodium and water intake are closely related, the difficulty has been to determine whether the observed responses to high salt intake are a result of the body fluid volume or sodium per se. Recent studies from our laboratory have shown that volume expansion is a prerequisite for the elevation of arterial pressure, which occurs with excess salt in two models of experimental hypertension (Dahl salt-sensitive rats and angiotensin II-induced hypertension). Future studies must determine how an expansion of blood volume can lead to sustained elevation of arterial vascular resistance. Two major theories have been advanced to explain these events. Whole body autoregulation proposes that with blood volume expansion, each tissue locally regulates blood flow toward a normal state, with a resultant increase in total peripheral vascular resistance. The possible mechanisms are not well understood. The second theory proposes that the expansion of blood volume is detected by stretch receptors that stimulate the release of a putative ouabainlike factor, which leads to inhibition of Na,K-ATPase activity in vascular smooth muscle and vasoconstriction. To date, nei-
ther theory has been demonstrated as the cause of increased systemic vascular resistance in volume-induced forms of hypertension.

In addition, future investigations must continue the search for the link between blood volume and arterial resistance, which will be a major challenge, because these mechanisms are among the most fundamental of those that link the metabolic needs of the tissues to the final regulation of blood flow. Technology may provide some new approaches to confront these problems in noninvasive ways. Nuclear magnetic resonance spectroscopy may provide useful approaches that require a relation of changes in regional metabolism and oxygen consumption to blood flow and vascular resistance in animals and human subjects that undergo increases of salt intake. Similarly, epifluorescent imaging techniques that use fluorescence-labeled plasma proteins or red blood cells can now be used with intravital microscopy to provide two- or three-dimensional characterization of capillary blood flow and distribution by means of implanted windows. Such techniques applied to the study of salt sensitivity could aid understanding of the basic signaling processes in situ between cells and vessels.

**Salt-Induced Structural Changes of the Vasculature**

Evidence has emerged that salt-sensitive forms of hypertension are associated with substantial structural changes of the vasculature. These changes include classically described increased wall-to-lumen ratio and more recently described reduction in the number of microvessels (rarefaction). Evidence now shows that such changes could contribute to the increased total peripheral vascular resistance in these forms of hypertension, but neither the quantitative significance nor the mechanisms are understood. Future research will require clarification of these issues. New and more reliable techniques for quantification and enumeration of microvessels should allow for progress in this field.

**Ion Transport Pathways**

Not much work has been done to characterize alterations of vascular smooth muscle ion transport in salt-sensitive experimental or human models of hypertension. Although vascular and endothelial cells are small and difficult to manage, some investigators have successfully applied whole-cell patch-clamp technology to study them. Single-channel patch-clamp techniques will be increasingly applied to the study of \( K^+ \) and \( Ca^{2+} \) channels in cells of humans and animals. These techniques, when combined with biochemistry studies of intracellular signaling pathways, should provide important new data on both primary and secondary changes of vessel reactivity and maintenance of enhanced vascular tone in hypertension. Such studies should also provide important basic information required for more rational drug designs.

There is evidence that endothelial cells, which line the inner surface of blood vessels, serve as mechanotransducers that influence the synthesis and secretion of a variety of vasoactive substances produced and released by these cells, such as prostacyclin, endothelium-derived relaxing and contracting factors, endothelin, prostacyclin, and other potential substances. The way in which salt intake influences these pathways and the effects of these substances on ion channel activity of vascular smooth muscle needs to be determined. These are important areas of future research, which will advance our understanding of the mechanisms that relate changes in blood volume to changes in vascular resistance.

**Role of Extravascular Interstitial Matrix**

The partition of extracellular fluid between vascular and interstitial compartments is determined by the balance of hydrostatic pressure and colloid osmotic (Starling) forces across the capillary membrane. Although the hydrostatic pressure and oncotic forces have been measured within the capillary circulation, the forces outside the capillary have remained difficult to quantify, and the understanding of the factors that determine the distribution of extracellular fluid and blood volume remains limited.

Recently, high-resolution intravital microspectrophotometric techniques have been developed to measure interstitial protein content. Measurements of perivascular protein concentrations in the loose connective tissue of the mesenteric circulation are now possible and require extension to other regions. The initial data showed that interstitial protein distribution is nonuniform, with the protein organized into periodic nonuniform ridges and valleys in nonvascular tissue regions. Clusters of proteins were observed along the walls of the microvessels and accompanied by radial gradients in protein distribution. High protein tunnels were occasionally seen that extended from a microvessel into the interstitial matrix.

Because interstitial protein homogeneity must be assumed for the Starling equilibrium equations, the implications of these new observations on the distribution of body fluids are considerable. Future application of this technology would not only greatly enhance our understanding of the basic mechanisms that control the partitioning of extracellular fluid but would also enable determination of the extent to which ionic factors such as sodium and calcium could alter the proteins of the interstitial matrix and excluded volume.

**Genetic Research**

One obvious area of future research is concerned with the genetic factors that predispose toward hypertension because of salt intake. The multiple and redundant mechanisms that participate in sodium and water homeostasis undoubtedly involve more than a single genetic site. Techniques of molecular biology could enable determination of the genetic expression of neural and endocrine controllers, membrane receptors, ion channels, and pump activity.
We have just begun to use the tools of molecular biology for multigenic disorders, and little progress has been made in defining the genetic basis of various types of hypertension. Molecular probes and in situ hybridization techniques are currently used primarily as improved bioassay systems to qualitatively describe and locate proteins and enzymes in various locations. The gap between the identification of new factors and the discovery of their function is widening at a distressing speed. Future investigations must proceed beyond this descriptive stage and apply these techniques to determine the quantitative and functional significance of these molecules.

The underlying genetic abnormalities that are responsible for the phenotypical expression of various known abnormalities in salt-sensitive forms of hypertension could be identified.

Genomic clones are frequently available to researchers interested in precisely locating genetic alterations on each chromosome. More complete libraries for the commonly studied organisms will hopefully become more available. Studies of the genetic linkage between different mutations can now be used to generate chromosome maps that provide the relative locations of the genes. Clones of the mapped genes can be used to identify genetic mutations in various forms of hypertension, using techniques such as chromosome walking. Alternatively, DNA probes can be used in hybridization reactions with RNA to find out whether various tissues and cells express important regulatory genes and how they are transcribed, spliced, and translated into final products. Characterization of gene regulatory proteins that help initiate DNA replication and control gene transcription is needed.

The ultimate test of altered gene function is to reinsert altered genes in the reproductive cell line of mammals and study their expression. Normal genes can be replaced with the altered ones or altered genes with normal ones. Production of these transgenic animals could provide powerful tools for the study of specific genetic alterations thought to confer salt sensitivity in an animal. Although the early techniques to accomplish this procedure have been rather crude because the investigator cannot control where the foreign gene will be inserted in the DNA of the host cell, recent approaches seem more promising. As more is learned about the nature of the controls that regulate the quantity, target specificity, and timing of the gene expression, exciting advances should be forthcoming.

Need for Both Cellular and Integrative Approaches

Arterial pressure regulation is one of the most complex integrative functions of higher organisms. When one considers future directions of research on salt and hypertension, it is apparent that no single approach will in itself explain the cause of the problem. An understanding of both the cellular and integrative aspects of the organism is essential. Exciting developments in cell and molecular biology can provide important new insights about regulatory function. But the development and testing of comprehensive hypotheses for blood pressure regulation is becoming more difficult as greater numbers of investigators work on smaller pieces of the system. Two needs must be met to achieve future progress in this field. First, it is necessary that equally imaginative whole animal and cell/molecular research be carried out. Second, increasing efforts must be made to merge and integrate the data obtained at the cellular level with that obtained in intact systems.

References

KEY WORDS: sodium • blood pressure • sodium-dependent hypertension
Salt and hypertension--future directions.
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Hypertension. 1991;17:I205
doi: 10.1161/01.HYP.17.1_Suppl.I205

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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World Wide Web at:
http://hyper.ahajournals.org/content/17/1_Suppl/I205

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