SUMMARY  In this review, postulated passive and active fluxes of sodium, potassium, and calcium across the sarcolemma of the normal vascular smooth muscle cell are first summarized. Some practical problems encountered in their measurement are also mentioned. The review then considers how these fluxes appear to be altered in various forms of hypertension in animals and humans. Emphasis is given to abnormal fluxes of sodium and potassium due to altered sodium pump activity and permeability. Increasing evidence indicates that sodium retention due to increased sodium intake or decreased sodium excretion causes hypertension by releasing a humoral pressor substance from brain. This substance, which may be the putative natriuretic hormone, inhibits Na⁺,K⁺-ATPase and sodium pump activities in blood vessels and heart, thereby increasing contractile activity. In the genetic models of hypertension, the primary defect appears to be increased permeability of the vascular smooth muscle cell wall to sodium; pump activity increases to compensate for the increased inward leak of sodium. This may also be the case in patients with heritable essential hypertension. The possible consequences of super-imposing the sodium pump inhibitor on the primary defect are also considered. This may occur when animals with genetic hypertension or patients with heritable essential hypertension retain sodium subsequent to increased sodium intake and/or decreased ability to excrete sodium. Such superimposition should raise intracellular sodium concentration to high levels since now the pump would not fully compensate for the increased inward leak of sodium. 

KEY WORDS  * vascular smooth muscle  •  sodium, potassium, calcium  •  low renin hypertension  •  genetic hypertension  •  sodium-potassium pump  •  permeability  •  natriuretic hormone

T HIS paper reviews the transport of sodium, potassium, and calcium across the sarcolemma of the vascular smooth muscle cells in various types of hypertension. The smooth muscle cells of major importance in hypertension are those in the resistance vessels, i.e., the arterioles. Since it is not convenient to study the arteriole itself, we frequently make inferences from studies in large vessels (animals) and from red and white blood cells (humans). This, of course, is not without danger since there is no assurance that transport processes in larger vessels and circulating blood cells are the same as they are in the arteriole; large vessels contain different proportions of cell types and extracellular substances and the circulating blood cells clearly are different morphologically.

Figure 1 presents schematically postulated fluxes in the normal cell.\(^1,2\) It shows that these ions move passively down their electrochemical gradients. The rates at which they move depend on the extent of their gradients and the ease with which they cross the sarcolemma (permeabilities). The latter are voltage-dependent. For example, the arrow for calcium represents total passive calcium influx down its electrochemical gradient, part of which is voltage dependent. Figure 1 also shows that the three ions move actively against their electrochemical gradients. In the steady state, active fluxes equal passive fluxes. The high concentration of potassium and low concentration of sodium in
intracellular fluid relative to extracellular fluid results to a large extent from the activity of the sodium-potassium pump in the sarcolemma. This pump actively transports potassium into and sodium out of the cell, and the pump is stimulated by the addition of potassium ions to extracellular fluid and of sodium ions to intracellular fluid. The pump is inhibited by lowering the extracellular potassium concentration or the intracellular sodium concentration. It is also inhibited by adding cardiac glycosides, such as ouabain, to the extracellular fluid.

The sensitivity of the pump to ouabain is used in estimating its activity. Thus, the active pumping of sodium or potassium is the difference between the flux in the absence and presence of enough ouabain to stop the pump (ouabain-sensitive flux). The flux with the pump stopped is passive (ouabain-insensitive flux) and, as pointed out above, depends on the electrochemical gradient and the permeability of the ion in question.

In studying the activity of the sodium pump in vascular smooth muscle, we excise a vessel from an animal and place it in an artificial fluid. This "depressurizes" the vessel and removes it from the internal environment, both of which might influence the pump rate. We hope that the vessels from hypertensive and normotensive animals are influenced equally. We sometimes make inferences from ions that do not occur naturally. For example, active transport of radioactive rubidium is frequently used to reflect active transport of potassium; rubidium is handled by the sodium-potassium pump like potassium, and radioactive rubidium has a lower energy emission and a longer half-life than radioactive potassium. We often measure the maximal rate of pumping (achieved with sodium loading) rather than that which occurs naturally because, among other things, the numbers are larger and therefore easier to measure. Thus, practical considerations, of necessity, help determine the study design and may affect its findings.

When cells are broken and the cell membranes isolated, an enzyme can be demonstrated that splits adenosine triphosphate. This enzyme is activated by sodium and potassium and hence is called Na\(^+\),K\(^-\)-ATPase. Like the sodium-potassium pump in the intact cell, the Na\(^+\),K\(^-\)-ATPase is inhibited by low concentrations of potassium or sodium and by exposure to ouabain. It is therefore thought that this enzyme occupies a central position in the operation of the pump. By splitting intracellular adenosine triphosphate, it provides the energy for potassium and sodium transport.

Unfortunately, it is not yet practical to measure Na\(^+\),K\(^-\)-ATPase activity in the sarcolemma of the vascular smooth muscle cell. We therefore frequently make inferences from Na\(^+\),K\(^-\)-ATPase activity of the heart where the sarcolemma is more easily isolated in a purer form and where the density of the Na\(^+\),K\(^-\)-ATPase molecules per unit membrane is greater. The density of the molecules can be estimated by ouabain binding, a technique that is just now being applied to hypertension.

As in many other cell types, the sodium-potassium pump in the vascular smooth muscle cell is electrogenic, i.e., it affects the membrane potential. Increased speed of pumping hyperpolarizes the membrane, whereas decreased speed of pumping depolarizes the membrane. These effects are thought to result from the transfer of the charge across the cell membrane due to unequal pumping rates for sodium and potassium (more sodium ions are normally transported out than potassium ions in per pump cycle). Thus, reduction of the pump speed by lowering the external potassium concentration or by administration of ouabain results in an accumulation of a net positive charge on the inside and, hence, depolarization. Since part of the total passive calcium influx down its electrochemical gradient is voltage-dependent, this should result in increased calcium influx.

Figure 1 postulates two modes of active calcium extrusion against its electrochemical gradient. One mode is via a calcium activated ATPase-pump system, as occurs in the red blood cell. Vascular smooth muscle membranes form vesicles that pump calcium; the problem is that these membranes are a mixture of sarcolemma and sarcoplasmic reticulum. The other mode is via a Na\(^+\)-Ca\(^+\) exchange mechanism. Here the efflux of calcium is dependent on the sodium gradient. For example, an increase in intracellular sodium concentration subsequent to slowing of the sodium-potassium pump would reduce the sodium gradient and hence the efflux of calcium, resulting in a rise in intracellular calcium concentration. The problem here is that some investigators question the presence of this mechanism in vascular smooth muscle.

This review will concentrate on abnormalities of active and passive sodium and potassium transport in the vascular smooth muscle of animals and humans with various forms of hypertension. We are, of course, interested in the pump activity and permeability of vascular smooth muscle in vivo. Since we can't study these parameters in vivo, we have three choices, all of which are now being used. We can: 1) remove the artery and place it in a foreign environment; 2) remove the environment and place it on a foreign artery; and 3) remove both the artery and the environment and bring them back together again in vitro. Method 1 (removing the artery and placing it in a foreign environment such as Krebs solution) has in two of the models of low renin hypertension generated contrasting results in different laboratories. This may well be explained by differences in protocols. For example, in the one-kidney-DOCA-saline model, Friedman and Nakashima\(^6\) and Jones\(^1\) found increased pump activity, whereas Pamnani et al.\(^12\) and Songu-Mize et al.\(^13\) found decreased pump activity. However, the protocols differed with respect to preincubation time, being longer in the studies of Jones and Friedman. Pamnani et al. puzzled over this and then repeated their studies,\(^14\) but this time, with some arteries, they interposed a long preincubation period before studying pump activity. As in the earlier study, those arteries examined immediately had reduced pump activity but those studied...
after preincubation did not. Perhaps a pump inhibitor leaches out and washes away with time of incubation. The advantage of prolonged incubation is that it allows concentration. Here, one applies plasma from the normotensive and hypertensive animal to pieces of the same normal artery, which should have the same structure and internal sodium concentration.

Method 3, placing the hypertensive artery in its own internal environment, should approach the in vivo situation most closely. We are just now exploring this option.

Sodium Pump, Natriuretic Hormone, and Hypertension

A substantial body of evidence indicates that sodium intake influences the level of arterial pressure, particularly when renal function is impaired. The reverse is also true. Impaired ability to excrete sodium can raise arterial pressure, particularly when sodium intake is increased. We here consider a possible mechanism by which sodium retention can elevate the blood pressure, i.e., via a circulating sodium pump inhibitor that may be a natriuretic hormone.

It has long been recognized that sodium excretion by the kidney is adjusted to keep total body sodium reasonably constant despite large variations in intake and in losses via skin and other routes. The mechanism whereby the kidney adjusts its excretion of sodium is very complex and only partially understood. However, many studies suggest that one component of this mechanism is a circulating sodium pump inhibitor that acts on the renal tubules to reduce reabsorption of sodium, i.e., a natriuretic hormone. According to this concept, sodium retention increases plasma volume; this stimulates "receptors" in the central circulation, which somehow results in the release of the humoral natriuretic substance from the brain.

This hypothesis is based on several lines of evidence. Cross circulation studies have been performed in which a recipient animal, or an isolated kidney, is perfused with blood from a donor animal. When the blood volume of the donor animal is acutely expanded, sodium excretion by the kidney of the recipient animal or by the isolated kidney increases. Isolation of the circulation to the head of the donor animal prevents the response. Plasma and urinary extracts from salt-loaded subjects and from subjects seated in water to the neck (to selectively expand the central circulation) increase renal sodium excretion by assay animals and inhibit sodium transport by epithelial membranes such as toad bladder. These same extracts inhibit renal Na⁺,K⁺-ATPase. Plasma extracts from saline expanded rats with a lesion of the anteroventral third ventricle (AV3V) are inactive on toad bladder. These studies suggest that the transferable natriuresis observed in the cross-circulation experiments is due to a circulating sodium pump inhibitor released from or influenced by the AV3V area of the brain.

Other lines of evidence suggest that this sodium pump inhibitor may be the link between salt retention and hypertension. According to this hypothesis, certain hypertensive subjects have a defect in sodium excretion, leading to high circulating levels of the sodium pump inhibitor. This causes hypertension by promoting constriction of the resistance vessels and by stimulating contractile activity in veins and heart.

A number of studies suggest the existence of a circulating vasoconstrictor and sensitizing agent in salt-dependent low-renin hypertension. Elevated pressure can be transferred from the animals with low-renin hypertension to assay animals by cross circulation of blood or through a parabiotic connection. Plasma from hypertensive animals and man causes a slow increase in blood pressure and increased pressor responses to vasoactive agents when injected into assay rats (for a review, see ref. 24). Salt-loading increases the level of this factor. The agent in the plasma is heat stable and appears to have a molecular weight of about 1000.

Animals with salt-sensitive low-renin hypertension have reduced sodium pump activity in their blood vessels (fig. 2) and reduced Na⁺,K⁺-ATPase activity in their hearts, and humans with essential hypertension have reduced sodium pump activity in their white blood cells. They also have elevated plasma levels of a sodium pump and Na⁺,K⁺-ATPase inhibitor, i.e., the vascular and white blood cell pump defects can be reproduced in normal blood vessels and white blood cells by applying plasma from the hypertensive animal or patient, and plasma from hypertensive patients reduces the activity of Na⁺,K⁺-ATPase obtained from kidney. In some studies the pump inhibitor is found in the plasma of most hypertensive patients, whereas in others, it is found mainly in those subjects with low plasma renin activity.

Is the pressor and sensitizing agent natriuretic hormone? Is the vascular and white blood cell pump inhibitor natriuretic hormone? All three activities are heat stable. All three activities result from a small molecule. Natriuretic hormone reduces toad bladder short circuit current and renal Na⁺,K⁺-ATPase activity; so does plasma obtained from hypertensive subjects. Hypertensive plasma has pressor and sensitizing activity; so does natriuretic hormone. An AV3V lesion eliminates natriuretic hormone in blood of volume expanded animals; it also reduces blood pressure and the blood level of the vascular sodium pump inhibitor in animals with low renin hypertension. The agent found in the plasma of animals with low renin hypertension does not appear to be vasopressin or atrial natriuretic factor.

An inhibitor of the sodium pump in blood vessels and heart might cause vasoconstriction and increased...
LOW RENIN
PRESUMABLY VOLUME EXPANDED FORMS OF HYPERTENSIONS

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<thead>
<tr>
<th>OUABAIN SENSITIVE 86Rb UPTAKE IN BLOOD VESSELS</th>
<th>OUABAIN INSENSITIVE 86Rb UPTAKE IN BLOOD VESSELS</th>
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<tr>
<td>1-KIDNEY, 1-WRAP HT DOGS</td>
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<td>1-KIDNEY, 1-CLIP HT RATS</td>
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<td>1-KIDNEY, DOCA-SALT HT RATS</td>
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GENETIC HYPERTENSIONS

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<td>R N.T. RATS ON HIGH SALT</td>
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Figure 2. Summary of findings in arteries of animals with various types of hypertension. Rb+ can replace K+ in transport studies; it is handled by the Na+-K+ pump like K+. Radioactive Rb+ is used instead of radioactive K+ because it has a lower energy emission and a longer half-life. Ouabain-sensitive 86Rb uptake reflects Na+-K+ pump activity. Ouabain-insensitive 86Rb uptake in part reflects the permeability of the cell membrane to 86Rb. Notice that ouabain-sensitive uptake is reduced in the low renin models of hypertension and that ouabain-insensitive uptake is elevated in the genetic and DOCA models of hypertension. HT = hypertensive, NT = normotensive.

myocardial contractile force by acting directly on the vascular smooth muscle and myocardial cells. The pump in vascular smooth muscle is electrogenic. Inhibition of the pump should cause depolarization and increased calcium influx. Depolarization has in fact been observed in one low renin model of hypertension. Alternatively, inhibition of the sodium pump decreases calcium efflux via the Na-Ca exchange mechanism. While these have been the most widely discussed theories to date, we some time ago suggested an indirect mechanism which may operate in series with the direct mechanism. Uptake of norepinephrine by adrenergic nerve endings is a Na+,K+-ATPase-dependent process. Inhibition of uptake should lead to increased concentration of norepinephrine in the cleft, vasoconstriction and hence hypertension. This would explain why the sympathetic nervous system appears to be activated in some low renin models of hypertension. It might also explain why endogenous norepinephrine content of heart and blood vessels is reduced in these same models. We have, in fact, extracted from canine plasma a heat stable small molecule which inhibits norepinephrine uptake by canine saphenous vein and increases its contractile response to electrical stimulation.

Is it paradoxical that a natriuretic substance causes hypertension? The answer is no, if one accepts the theory that some forms of hypertension occur when a defect in renal sodium excretion causes increased plasma levels of an agent which causes both decreased tubular sodium reabsorption and increased contractile activity of heart and blood vessels. Increased pressure and decreased reabsorption would be the best way to rid the body of the excess sodium and water. Accordingly, the hypertension could be viewed as an adaptive mechanism designed to maintain normal extracellular fluid volume; it is the price we pay for volume homeostasis.

Not all observations are compatible with the view that salt induced hypertension results from a circulat-
ing inhibitor of the sodium pump. The digitalis glycosides have been used clinically for centuries, with little documented evidence that they raise arterial blood pressure. On the other hand, ouabain causes increased blood pressure due entirely to an increase in total peripheral resistance in normal human subjects and in the normal unanesthetized dog. It also increases pressure in the normal pentobarbital-anesthetized dog, and the response is amplified when the antagonistic effect of diuresis is nullified. Thus, the digitalis glycosides may not cause hypertension clinically because they are usually given to patients with failing hearts and normal renal function.

Another problem with the natriuretic hormone theory is that decreased pump activity is not observed by all investigators in the low renin models of hypertension. This apparent discrepancy is addressed in the first and third sections of this review.

A third problem with the theory is that the biochemical structure of natriuretic hormone remains unknown. Some investigators believe it is a small peptide, while others deny this. It is possible that there are both peptide and non-peptide digitalis-like substances which play a role in the regulation of both sodium homeostasis and cardiovascular function. Of interest is the recent observation that a heptapeptide fragment of ACTH (amino acids 4 through 10) has many, but not all, of the properties of natriuretic hormone.

Further work in this field might lead to advances in the management of hypertension. For example, antibodies to digoxin lower the blood pressure in the rat with one-kidney, DOCA, saline hypertension. It is possible that a new class of antihypertensive drugs will emerge from development of antagonists to the vascular actions of natriuretic hormone.

**Sodium Permeability and Hypertension**

Many studies suggest that, in some forms of hypertension, sodium transport across cell walls is abnormal because of increased permeability to the sodium ion rather than from a suppressed sodium pump, i.e., sodium accumulates in the cell because of increased influx due to a leaky cell membrane rather than from decreased efflux due to a suppressed sodium pump. One way this becomes apparent is to study monovalent ion transport after stopping the sodium pump with high concentrations of the cardiac glycosides, most commonly ouabain.

In animals, such studies can be conducted in blood vessels. Arteries and veins are taken from animals with various types of hypertension, placed in an artificial extracellular fluid containing ouabain, and monovalent ion transport then studied. Such studies provide evidence for increased permeability of the vascular smooth muscle sarcolemma to sodium and other monovalent ions in the genetic (spontaneously hypertensive rat; Dahl salt-sensitive rat) and ouabain-insensitive defects appear to be acquired and related to salt intake and excretion, renin level, and a humoral sodium pump inhibitor, as pointed out in the preceding section.

The ouabain-sensitive defects in humans have been mainly described in red blood cells (by contrast, the ouabain-sensitive pump defects have been mainly described in white blood cells where the sodium pump is more active). One test, used by Canessa et al. relies on the measurement of Na⁺-driven efflux of Li⁺ from Li⁺-loaded, ouabain-treated red blood cells (Na⁺-Li⁺ countertransport). In this test, most patients with essential hypertension have Na⁺-driven Li⁺ efflux, which is about twice as rapid as normal. Another more simple and direct technique described by Mahoney et al. measures Na⁺ accumulation by red blood cells in a solution containing ouabain; Na⁺ uptake by red blood cells from patients with essential hypertension averages twice normal whereas it is not elevated in patients with secondary hypertension and no family history of essential hypertension. Garay and
Meyer\textsuperscript{a} did much to call our attention to the heritability of abnormal ouabain-insensitive sodium and potassium fluxes in the erythrocytes of patients with essential hypertension.

While it is not without danger to extrapolate the findings in blood cells to vascular smooth muscle cells, these findings suggest that we may eventually find in the blood vessels of hypertensive humans the same categories of defects observed in the blood vessels of animal models of hypertension. These defects are: 1) a genetically determined increase in the permeability to sodium, as observed in the spontaneously hypertensive rat; 2) an acquired decrease in sodium pump activity, as observed in the low renin models of hypertension; 3) a genetically determined increase in sodium permeability upon which is superimposed an acquired sodium pump defect, as probably occurs when the Dahl salt-sensitive rat is placed on a high-salt diet; and 4) an acquired increase in sodium permeability and an acquired sodium pump defect, as observed in the rat with one-kidney-DOCA-saline hypertension. Some clinical counterparts might be, respectively, 1) heritable essential hypertension with normal salt intake and renal function; 2) renal disease with normal- or high-salt intake; 3) heritable essential hypertension with high-salt intake or decreased renal function; and 4) primary aldosteronism.

Sodium pump activity might be increased in some categories and decreased in others. For example, it might be increased in heritable essential hypertension, in response to the increased inward leak of sodium, and decreased in renal disease, in response to the humoral sodium pump inhibitor. Pump activity might even be elevated in the presence of the inhibitor if the inhibitor is superimposed on an increase in permeability, which increases the inward leak of sodium, as in heritable essential hypertension with a high-salt intake or decreased renal function. Common to all, however, would be increased intracellular sodium concentration and consequently increased intracellular calcium concentration.

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