Summary: This review considers in some detail the hypothetical relationships between sodium fluxes, both active and passive, across the cell membrane, and intracellular sodium concentration in vascular smooth muscle in the animal models of hypertension. It appears that two basic types of transport defects, increased cell membrane permeability to sodium and decreased active pumping of sodium at a given internal sodium concentration, can exist in vascular smooth muscle in experimental hypertension, and that sometimes the two defects coexist, further increasing internal sodium concentration. It is possible that eventually we may find similar transport defects in vascular smooth muscle in humans with arterial hypertension. Decreased active pumping at a given internal sodium concentration appears to result from a humoral sodium pump inhibitor. Future directions for research in the area are also considered. First priority should be given efforts to determine the chemical structure of the sodium pump inhibitor(s). High priority should also be given to attempts to measure passive and active sodium fluxes and intracellular sodium concentration in vascular smooth muscle cells in vivo, and to determine the role of atrial natriuretic factor in the genesis and maintenance of hypertension.

Key Words: sodium and potassium flux, sodium and potassium permeability, sodium-potassium pump, humoral sodium pump inhibitor, atrial natriuretic factor, vascular smooth muscle, experimental hypertension

Studies during the last two decades suggest that two basic types of transport defects can exist in the vascular smooth muscle cell in experimental hypertension, and that, under certain circumstances, the two defects can coexist and amplify their individual effects on cell sodium concentration. The first seems to be characteristic of the genetic and mineralocorticoid models of hypertension and the second appears to be characteristic of experimental low renin hypertension resulting from impaired ability to excrete salt. Decreased active pumping at a given intracellular sodium concentration appears to result from a humoral sodium pump inhibitor, possibly released from the hypothalamus. These two defects may coexist when an animal with genetic hypertension has defective renal function with respect to sodium excretion, particularly when sodium intake is increased, or when plasma mineralocorticoid levels are high. Coexistence would drive the intracellular sodium concentration to higher levels, further increasing contractile activity of vascular smooth muscle.

This review considers hypothetical relationships between sodium fluxes across the cell membrane and intracellular sodium concentration in vascular smooth muscle in the animal models of hypertension. Future directions for investigation in the area are also suggested.

Ion Transport in Vascular Smooth Muscle in Experimental Hypertension

In a previous review we addressed passive and active fluxes of sodium across the sarcolemma of the normal vascular smooth muscle cell. We postulated that sodium moves passively into the cell along its electrochemical gradient and actively out of the cell by way of the sodium-potassium pump, and that, in the steady state, active flux equals passive flux. We also postulated that the sodium-potassium pump is electrogenic, with a decrease in pump activity causing depolarization. Figure 1 presents hypothetical curves re-
Figure 1. Hypothetical curves showing passive sodium influx (leak flux) and active sodium efflux (pump flux) as a function of the intracellular sodium concentration. Solid curves represent normal relationships and dashed curves represent relationships in which the permeability to sodium is increased and maximal pump velocity ($V_{\text{max}}$): (substrates, except for sodium, assumed to be at saturating values) is either increased or decreased. Points a through f represent six different steady states (pump flux equals leak flux) at which intracellular sodium concentration is constant with time. Point a is the normal steady state and points b through f represent five different abnormal steady states. An increase in membrane permeability to sodium ($P$) would result in the establishment of a new steady state (b) at a higher intracellular sodium concentration ($C_i$). If pump inhibition occurs ($V_{\text{max}}$ decreased, $P$ normal), a new steady state will also be established (c) at a higher internal sodium concentration ($C_i$). If $P$ were increased and $V_{\text{max}}$ decreased simultaneously, a new steady state would be established (d) at an even higher intracellular sodium concentration ($C_i$). The case where $V_{\text{max}}$ is increased is also illustrated, but a permeability decrease is not, as we know of no model of experimental hypertension with this abnormality. Modified from Clough et al. \(^{50}\)

Lating passive sodium influx (leak flux) and active sodium efflux (pump flux) to intracellular sodium concentration in the vascular smooth muscle cell of the normal and hypertensive animal, modified from Clough et al. \(^{50}\) Pump flux is defined as the difference between flux in the absence and presence of ouabain (ouabain-sensitive flux) and leak flux is defined as the flux in the presence of ouabain (ouabain-insensitive flux). (As so defined, all modes of sodium transport that are not sensitive to ouabain are lumped together; these modes could include sodium-potassium cotransport, sodium-hydrogen exchange, sodium-calcium exchange, passive sodium leak by way of sodium-specific and -nonspecific channels, and perhaps others, all less well defined in vascular smooth muscle than in red blood cells, for example.) For simplification, all pump substrates except sodium are assumed to be present at saturating concentrations; that is, the pump is operating maximally at each sodium concentration. In the steady state, active pump efflux equals the net passive gradient-dependent influx, and therefore intracellular sodium concentration remains relatively constant. In Figure 1 the normal active and passive fluxes are represented by solid lines and the steady state (point a) occurs where the two lines intersect.

As noted previously, \(^{8}\) if there is a sufficiently large increase in the permeability of the sarcolemma of the vascular smooth muscle cell to sodium, the sodium leak flux will exceed the sodium pump flux. If this imbalance is not compensated by a sufficiently large increase in the maximal velocity of the pump ($V_{\text{max}}$), then a new steady state will be reached at a higher intracellular sodium concentration. This is illustrated in Figure 1, where $V_{\text{max}}$ is normal and sodium permeability ($P$) is increased (point b, $C_i$). Data in the literature suggest that the transport defect in the vascular smooth muscle cell of the Okamoto spontaneously hypertensive rat (SHR) is of this type. Both ouabain-sensitive and sensitive fluxes are increased in blood vessels\(^{5,7}\) and Na\(^+\),K\(^+\)-adenosine triphosphatase (ATPase) activity is normal\(^{51}\) or decreased in cardiac membranes.\(^{52}\) Ouabain binding is normal in caudal artery\(^{53}\) and decreased in cardiac membranes.\(^{52}\) The membrane of the vascular smooth muscle cell is depolarized in vivo but not in vitro at $37^\circ C$ (the diffusional component of the membrane potential is decreased and the electrogenic pump component of the membrane potential is increased in vitro).\(^{54}\)

On the other hand, if the $V_{\text{max}}$ of the sodium-potassium pump of the vascular smooth muscle cell decreases, the sodium pump flux will be less than the leak flux. If this imbalance is not compensated by a decrease in the permeability of the sarcolemma to sodium, then a new steady state will be reached at a higher intracellular sodium concentration. The end effect is similar to that of increased permeability, that is, a net accumulation of intracellular sodium. This is illustrated in Figure 1 where permeability is normal and $V_{\text{max}}$ is decreased (point c, $C_i$). Data in the literature suggest that the transport defect in the vascular smooth muscle cell of the dog with one-kidney, one wrapped hypertension and the rat with one-kidney, one clip or reduced renal mass-saline hypertension is of this type. Ouabain-sensitive $^{86}$Rb flux is decreased but ouabain-insensitive $^{86}$Rb flux is normal in blood vessels,\(^{10-14}\) and Na\(^+\),K\(^+\)-ATPase activity is decreased in rat cardiac membranes.\(^{13,14,55,56}\) Ouabain binding appears to be decreased in cardiac microsomes from dogs with one-kidney, one wrapped hypertension\(^{57}\) and normal in aortic strips from rabbits with one-kidney, one clip hypertension.\(^{58}\) Bioassay evidence suggests that the decreased pump and Na\(^+\),K\(^+\)-ATPase activities result from a humoral sodium pump inhibitor.\(^{11-15,20,26,27}\) In the case of the one-kidney, one clip model, the membrane of the vascular smooth muscle cell is depolarized both in vivo\(^{59}\) and in vitro,\(^{20}\) and plasma supernates from these hypertensive rats depolarize vascular smooth muscle cells from normotensive rats.\(^{59}\) In reduced renal mass-saline hypertension, the arterial smooth muscle membrane is depolarized in vivo,\(^{60}\) and supernates from these rats depolarize vascular smooth
muscle cells from normotensive rats, but the membrane of arterial smooth muscle cells from the hypertensive rats is not depolarized in vitro.

If sodium permeability were increased and \( V_{\text{max}} \) decreased simultaneously, a new steady state would be established (point d) at an even higher intracellular sodium concentration (C5). Data from our laboratory and those of Songu-Mize et al. suggest that the transport defects in the vascular smooth muscle cells of the rat with one-kidney, deoxycorticosterone acetate (DOCA)-saline hypertension are of these types 5 to 8 weeks after the onset of DOCA-saline treatment. During this period, ouabain-insensitive \(^{86}\)Rb uptake is increased and ouabain-sensitive \(^{86}\)Rb uptake is decreased in arteries. \( Na^+,K^+\)-ATPase activity is decreased in cardiac microsomes, and the plasma has increased sodium pump inhibitory activity. These findings are compatible with increased permeability to sodium (possibly due to a direct effect of DOCA) and decreased \( V_{\text{max}} \) (due to an indirect effect of DOCA through a humoral sodium pump inhibitor). Ouabain binding by arteries has also been reported to be decreased during the seventh and eighth weeks. However, the membrane potential of the vascular smooth muscle cells in the caudal artery has been reported to be normal at Week 6. The arteries were in vitro at the time of measurement; perhaps both the DOCA and the inhibitor had been washed away.

Songu-Mize et al. found arterial ouabain-sensitive \(^{86}\)Rb uptake also reduced after 6 days of treatment with DOCA-saline, and Metzler et al. found, by radioimmunoassay, elevated plasma levels of a digoxinlike substance after 5 days of DOCA-saline treatment. More recently, Songu-Mize et al. obtained a positive plasma bioassay for a vascular sodium pump inhibitor on the sixth day. Thus, the findings during the fifth to sixth day are also compatible with decreased \( V_{\text{max}} \) due to a circulating sodium pump inhibitor. Membrane potential has not been measured at this time.

On the other hand, data from Songu-Mize et al. and other laboratories suggest that at intermediate times (9–28 days) after the onset of DOCA-salt treatment the pump flux is normal or increased. Furthermore, the radioimmunoassay for digoxinlike substance and the bioassay for vascular sodium pump inhibitory activity have been reported to be normal on the 11th and 28th days, respectively. Membrane potential has not been measured during this intermediate period. It has been suggested that aldosterone and DOCA can increase the biosynthetic rate of Na+,K+-ATPase molecules. Studies in the urinary bladder of the toad suggest that this effect follows an early response during which sodium transport increases rapidly and total tissue resistance falls concomitantly. The increased biosynthetic rate is associated with a further increase in sodium transport without a significant change in electrical resistance. Thus it appears that increased permeability precedes increased biosynthesis.

Perhaps time-dependent changes in the plasma level of the inhibitor and in the number of pump sites allow the steady state to occur at almost any point on the increased permeability curve (see Figure 1), in which case, pump flux can be decreased (point d), normal, or increased (points b and f), and internal sodium concentration can be normal (point f) or increased (points b and d).

Finally, if the \( V_{\text{max}} \) of the sodium-potassium pump of the vascular smooth muscle cell increases, the sodium pump flux will be greater than the leak flux. If this imbalance is not compensated for by an increase in the permeability of the sarcolemma to sodium, then a new steady state will be reached at a lower intracellular sodium concentration. This is illustrated in Figure 1, where permeability is normal and \( V_{\text{max}} \) is increased (point e, C5). The transport defect in the vascular smooth muscle cell of the rat with dexamethasone (glucocorticoid) hypertension appears to be of this type. Ouabain-sensitive flux is increased but ouabain-insensitive flux is normal. Glucocorticoids are known to induce synthesis of Na+,K+-ATPase in certain tissues. The level of the circulating pump inhibitor and membrane potential have not been measured in this model. It is not easy to see how increased pump flux in the absence of an increased leak flux could be relevant to the genesis of the hypertension.

Another possible case, namely, decreased permeability to sodium, is not illustrated, since we are not aware of a hypertensive model with this defect.

It is clear from Figure 1 that intracellular sodium concentration may be elevated with steady state sodium-potassium pump activity decreased (point c), increased (point b), or near normal (point d). The important determinant of intracellular sodium concentration is clearly the relationship between active influx and passive influx. It is also apparent that sodium-potassium pump activity may be decreased, increased, or normal in experimental hypertension, depending upon the contribution of permeability, the inhibitor, and the number of pump sites. Pump activity may be depressed or increased in the presence of the inhibitor. The presence of the inhibitor, therefore, cannot be inferred from pump activity; we must assay the plasma if we wish to know if it is present or absent. Finally, pump artifacts can occur if, during isolation of the vessel, permeability is increased or the inhibitor is washed away.

While we emphasize vascular smooth muscle in this review, it is possible that the inhibitor also affects the function of other tissues that influence blood pressure, notably myocardium and adrenergic nerve terminals. With respect to the latter, we have suggested that the inhibitor decreases norepinephrine uptake into cardiovascular adrenergic nerve terminals, thereby contributing to the hypertension. Plasma from the normal anesthetized dog in fact contains a substance that inhibits norepinephrine uptake by isolated saphenous vein.

Relevance to Hypertension in Humans

In 1983 we presented a tentative classification of arterial hypertension according to the heritability and ouabain sensitivity of the monovalent cation transport...
defects. In brief, we suggested that we may eventually find in the blood vessels of hypertensive humans the same categories of transport defects as are observed in animals: 1) genetically determined, ouabain-insensitive transport defects, as observed in SHR; 2) acquired ouabain-sensitive defects, as observed in the low renin models of hypertension; 3) genetically determined ouabain-insensitive transport defects on which is superimposed an acquired ouabain-sensitive transport defect, as probably occurs when the SHR or Dahl salt-sensitive rat is placed on a high salt diet; and 4) acquired ouabain-insensitive and ouabain-sensitive transport defects, as observed in the rat with one-kidney, DOCA-salt hypertension. Some clinical counterparts in humans might be, respectively, 1) heritable essential hypertension with normal salt intake and renal excretory function (see Figure 1, point b), 2) renal disease or latent renal dysfunction with normal or high salt intake (point c), 3) heritable essential hypertension with high salt intake or decreased renal excretory function (point d), and 4) primary aldosteronism (point d).

**Future Directions**

First priority should be given to efforts to determine the chemical structure and physiological actions of the sodium pump inhibitor(s). High priority should also be given to attempts to measure passive and active sodium fluxes and intracellular sodium concentration in vascular smooth muscle cells in vivo. Practical problems may delay in vivo measurements of passive and active fluxes, but in vivo measurements of membrane potential and intracellular sodium activity are feasible now. Sodium activity has been measured in ureteral smooth muscle cells in vitro, and membrane potential has been measured in arterial smooth muscle cells in vivo both in normotensive and hypertensive animals. Therefore, it should now be possible to measure sodium activity in arterial smooth muscle cells in vivo in normal and hypertensive animals.

A number of other areas also deserve attention. We should determine whether chronic inhibition of the sodium-potassium pump in blood vessels affects the number of pump sites in the sarcolemma of the vascular smooth muscle cell. Studies of other cells in vitro suggest that prolonged pump suppression with ouabain or low potassium increases ouabain binding, but the reverse has also been reported. On the other hand, weeks of treatment of dogs with digoxin failed to produce changes in the heart suggesting either up- or down-regulation of the enzyme.

We should also determine whether aldosterone and DOCA increase sodium permeability and the biosynthetic rate of Na+,K+-ATPase in vascular smooth muscle, as appears to be the case in certain other cells such as those in the toad urinary bladder (where increased permeability precedes increased biosynthesis). Enhanced biosynthesis has been attributed to a direct effect or to an indirect effect through an increase in intracellular sodium concentration. Thus, we should also determine whether prolonged increase in intracellular sodium concentration (achieved by increasing passive flux, decreasing pump flux, or both) increases the number of pump sites in vascular smooth muscle. Perhaps relevant are published measurements of ouabain binding by blood vessels and heart of animals with various types of experimental hypertension, since they are thought to have a long-term increase in intracellular sodium concentration. Data from four models of hypertension are now available, and in none was increased ouabain binding (or Na+,K+-ATPase activity) reported. In fact, decreased binding has been reported in SHR and rats with one-kidney, DOCA-salt hypertension, and dogs with one-kidney, one wrapped hypertension.

In a 1978 review we summarized several areas in the literature that suggest the potential for pressure-independent structural changes in heart and blood vessels in hypertension. These areas included 1) renal growth in hypertension, 2) renotropins and compensatory renal growth, and 3) mitogens (such as platelet-derived growth factor) and vascular smooth muscle cell proliferation. We concluded that consideration should be given to the possibility that pressure-independent hypertrophy and hyperplasia may occur in blood vessels in volume-expanded hypertension, and that an effort should be made to determine whether the natriuretic and renotropic factors are related and whether renotropin also acts on blood vessels to cause hypertrophy and hyperplasia. In a more recent review we said that findings since 1978 reinforce the need for such studies; the renotropins and vascular smooth muscle mitogens (platelet-derived growth, endothelial cell-derived growth and growth-inhibitory factors) are now better established and characterized, and cellular influx of sodium appears to influence the proliferation of certain types of cells. Mitogen-stimulated ion fluxes, particularly an amiloride-sensitive Na+-H+ exchange pathway, have been identified and proposed to be potential initial triggers for DNA synthesis in a variety of cells, including vascular smooth muscle cells. Decreased potassium concentration in culture medium augments kidney epithelial cell growth. We should encourage more studies of vascular smooth muscle cells, particularly those derived from SHR where there is evidence for increased sodium permeability, intrinsic growth rate, and growth stimulation by sodium.

We must examine the role of atrial natriuretic factor (ANF) in the genesis and maintenance of hypertension and determine if and how it interacts with the humoral sodium pump inhibitor. Hirata et al. showed that the hypertensive Dahl salt-sensitive rat has increased extractable tissue ANF but that its kidneys are hypore sponsive to exogenous ANF. We and Snajdar and Rapp confirmed these findings. We recently showed that extractable ANF is also increased in the reduced renal mass–saline hypertensive rat and that this disappears on reversing the hypertension. Extractable ANF also appears to be elevated in DOCA-saline-treated rats but decreased in SHR. These findings are currently being scrutinized by radioimmunoassay.
of ANF in atrial extracts.\(^\text{87, 91, 92}\) Perhaps, by atrial pressure, the ANF system compensates for increased extracellular fluid volume (due to reduced renal function, increased salt intake, or both), but the compensation is not complete in the low renin models of hypertension because of excessive fluid retention, defective ANF release, or kidneys that are hyporesponsive with respect to ANF (the plasma level of the sodium pump inhibitor would therefore remain elevated). We will need plasma levels of ANF in the various models in which atrial tissue levels are available to solve these problems, since tissue ANF levels need not reflect plasma levels. We must determine whether circulating ANF is in fact vasodilator\(^\text{86}\) as well as natriuretic and diuretic.

Antibodies to digoxin lower the blood pressure in rats with one-kidney, DOCA-saline\(^\text{44}\) and chronic aortic coarctation\(^\text{95}\) hypertension, models of low renin hypertension. Canrenone, a competitive antagonist of \([\text{Na}^+]_o K^+\)-ATPase,\(^\text{85}\) lowers blood pressure in rats with angiotensin-salt hypertension, a volume-expanded form, without causing natriuresis-diuresis.\(^\text{97}\) These findings deserve further exploration. So does the observation that 6-iodo-amiloride, a sodium channel blocker, can produce an immediate, large, sustained fall in blood pressure independent of excretory function in SHR,\(^\text{86}\) a model thought to be characterized by increased permeability of the smooth muscle cells to sodium.\(^\text{3, 6, 7}\)

If, in fact, increased permeability of the vascular smooth muscle cell to sodium and decreased active pumping at a given internal sodium concentration (due to the presence of the sodium pump inhibitor) sometimes coexist, then the aim of antihypertensive therapy in these cases should be to correct both defects. In theory, this might be accomplished by blockade of sodium channels in both the arteriole and renal tubule: blockade at the arteriole would correct the first defect and blockade at the tubule would correct the second by reducing blood volume and hence the plasma concentration of the volume-dependent inhibitor. Amiloride is a sodium channel blocker in epithelial tissues, but some of its analogues are better blockers in certain test systems and have not yet been evaluated as antihypertensive agents. 6-Iodo-amiloride has vasodilator as well as natriuretic activity and, as pointed out above, promptly reduces blood pressure in SHR. This genetic model of hypertension deserves more study with respect to the amiloride analogues because here sodium channel blockade may be specific therapy at two levels. Evidence from the literature indicates that SHR, in addition to having abnormally permeable vascular smooth muscle cells with respect to sodium, reabsorb sodium at an increased rate.\(^\text{95, 100}\) The cause of the latter is unknown but it could be due to an increased permeability of the luminal membrane to sodium, in which case, sodium channel blockade would also be specific therapy at the tubular level. Certain findings suggest that increased tubular reabsorption of sodium also occurs in the Milan strain of spontaneously hypertensive rat\(^\text{101}\) and in hypertensive humans.\(^\text{102}\)

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