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VASCULAR SMOOTH MUSCLE MEMBRANE IN HYPERTENSION

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INTRODUCTION

The title of this article has implications that differ greatly according to the reader's perspective: The clinician needs to know what the cell membrane has to do with elevated arterial pressure; the pharmacologist would like to know about functional abnormalities in this membrane; and the basic biologist is curious about whether these functional abnormalities can be accounted for by known differences at a molecular level. In an attempt to be global in our appeal, we have tried to address each of these three concerns.

The elevated arterial pressure of hypertension is usually caused by an increased total peripheral resistance, and the magnitude of a pressor response to a standard pressor stimulus is uniformly greater than normal in hypertension. For both theoretical and applied reasons, it is important to know whether there are mechanistic differences responsible for these abnormalities among the various types of hypertension.

At one level, all types of hypertension are the same in that in all of them arterial pressure and vascular responsiveness are elevated. Yet, as is evident in experimental hypertension, the initiating factors for genetic, renal, and mineralocorticoid hypertensions are entirely different. The relevant question is the relationship of sequences of events leading from the initiating factors to the final common outcomes. Where do these events converge? From the point of view of the current review it is important to ask whether all types of hypertension result from the same vascular changes. At one level this question can be given a convincing affirmative answer. In all types of hypertension, resistance vessels have walls that are thicker than normal. Folkow (1) has established that this characteristic not only increases vascular resistance by

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structural encroachment on the lumen, but also amplifies all pressor responses caused by vascular smooth muscle contraction. Further, Folkow (2) has demonstrated that this is an adaptive change in which the vessel wall thickens in response to the increase in wall stress characteristic of hypertension. Its development has the characteristics of a positive feedback system. Although it is certain that an increase in vessel wall stress can cause this thickening, more recent studies have suggested that in hypertension vessel wall hypertrophy may occur in the absence of an increase in wall stress. Owens & Schwartz (3) have described excessive numbers of polyploid cells in vessels of "pre-hypertensive" spontaneously hypertensive rats (SHR). Bell & Overbeck (4) have observed vessel wall hypertrophy in the normotensive portion of the vasculature below the aortic coarctation in rats that were hypertensive above the coarctation.

We need to ask whether the functional abnormalities of the vascular smooth muscle membrane we think are responsible for the vascular smooth muscle hyperresponsiveness are similar in all types of hypertension. Although we are not aware of a definitive comparative study, important abnormalities in the vascular smooth muscle function are evidently similar in several types of hypertension. These abnormalities, described in detail in the following sections, include: (*a*) increased sensitivity to various agonists, especially serotonin; (*b*) deficit in plasma membrane binding of calcium; and (*c*) increased membrane permeability to sodium, potassium, and calcium.

The calcium ion plays an essential role in initiating and regulating biological processes. For this reason the determinants of the calcium concentration at the site of the regulated process are critical to the normal performance of the process. In hypertension, the contractile process of vascular smooth muscle is not normal but excessive, suggesting that there is a problem either in the calcium regulation of this process or in the regulation of the calcium concentrations. At intracellular concentrations less than 0.1 μM, no contractile activity occurs in this muscle. Higher concentrations of calcium activate contractions as a direct function of the calcium concentration and reach maximum contraction at a calcium concentration of approximately 10 μM. The processes that regulate this calcium concentration therefore control vascular smooth muscle contraction. It is interesting that these regulatory processes are also significantly controlled by calcium. Both the actual contractile process and the processes that regulate intracellular calcium concentration are mediated by calcium binding proteins. The fault in the contractile process of vascular smooth muscle that is responsible for hypertension appears to be in calcium binding protein that regulates calcium concentration rather than in the protein that regulates contraction.

The contractile protein system of vascular smooth muscle is "myosin activated." That is, it is activated by the phosphorylation of a myosin light

chain. The phosphorylation is carried out by the enzyme myosin light chain kinase, which is activated by calcium and the calcium binding protein calmodulin. Although the contractile proteins appear to be normal in the hypertensive animals (5–8), there may be some abnormalities in this intracellular regulatory system (8). In studies of skinned aortic smooth muscle, Rinaldi et al (8) have found that the regulatory system is less sensitive to calcium in SHR than in Wistar Kyoto normotensive rats (WKY). They also found that this intracellular contractile system was more sensitive to the inhibitory effect of the calcium channel blocker nifedipine.

It is evident from this review that the major differences between vascular smooth muscle of normotensive and hypertensive animals reside in its plasma membrane.

VASCULAR SMOOTH MUSCLE SENSITIVITY

Many studies have demonstrated that vascular responsiveness to various stimuli is altered in hypertension (see 9–12 for reviews of their findings). The major observations are that hypertension is characterized by an increased sensitivity to constrictor stimuli, whereas vasodilator responsiveness is attenuated. These changes in the vasculature often precede or parallel the development of hypertension and are not the product of elevated blood pressure per se (13–16). Nor are the changes necessarily related to the initial state of vasoconstriction (17). It should be noted that the results of different studies vary greatly. For example, isolated aortic strips from mineralocorticoid hypertensive and two-kidney–one-clip, renal hypertensive rats are more sensitive to the contractile effects of arachidonate, i.e. have a lower threshold of response, than do aortic strips from normotensive rats (18). In contrast, aortic strips from SHR and psychosocial hypertensive mice did not demonstrate an increased sensitivity to the contractile effects of arachidonate in comparison with aortic strips from the corresponding normotensive controls. The findings of studies of responsiveness, in hypertension, to other vasoconstrictors (norepinephrine, angiotensin II, etc) and vasodilators (acetylcholine, nitroprusside, etc) show similar differences (see 9–12 for a review).

Part of the variation in research findings may be related to the fact that the blood vessel wall contains two other components (endothelium and nerve endings) that can alter the contractile state of the smooth muscle cells. The endothelium releases both dilator and constrictor factors in response to various stimuli. Several recent studies have demonstrated that these endothelial functions are altered in hypertension. Endothelium-dependent relaxation in response to acetylcholine, bradykinin, histamine, and A23187 is reduced in some blood vessels isolated from adult, genetically hypertensive rats (19–25). However, this reduction in the dilating influence of the endothelium does not

appear to be an initiating factor for elevated blood pressure. Reactivity to endothelium-dependent vasodilators in young SHR does not differ from that in age-matched normotensive rats, even though blood pressure is elevated in the young SHR. Furthermore, some blood vessels isolated from adult SHR show normal endothelium-dependent relaxation responses, while depressor responses to acetylcholine are exaggerated in the intact SHR as compared to normotensive WKY (24, 26). In the psychosocial hypertensive mouse, relaxation in aortic strips in response to acetylcholine is increased. This effect suggests that the endothelium may make a compensatory response that masks altered smooth muscle sensitivity to some vasoactive agents (27). Observations suggesting the possibility of a compensatory response by the endothelium have also been reported for aortic segments from SHR (24).

Recent studies suggest that the endothelium of arteries from SHR release contractile factors in response to some stimuli. Luscher & Vanhoutte (21) reported that acetylcholine causes endothelium-dependent contractions in isolated aortic segments from SHR but not in aortic segments from WKY. These contractile responses to acetylcholine were blocked by inhibitors of cyclooxygenase, thromboxane synthetase, and leukotriene synthetase. It appears that a muscarinic activation in the endothelium of SHR releases a cyclooxygenase product that causes contraction of aortic smooth muscle. Similar observations have been reported for serotonin-induced constriction in the coronary vasculature of SHR (28).

The activity of the adrenergic nerves in the blood vessel wall can influence the responsiveness of the smooth muscle cells (29). Several investigators have provided evidence that the neuronal uptake process in arteries from SHR is augmented in comparison with that in arteries from normotensive rats (30–34). This increased neuronal uptake process masks the increased sensitivity to catecholamines of the smooth muscle cells in blood vessels from hypertensive animals inasmuch as it removes the agonist from the vicinity of the smooth muscle receptors.

One of the unique features of the increased vascular sensitivity characteristic of the hypertensive state is that it is not the same for all stimuli. For example, contractile sensitivity to serotonin and methysergide is augmented to a greater degree in arteries from hypertensive animals than is contractile sensitivity to norepinephrine (see 35 for review). Arteries from hypertensive rats also show an unusual contractile sensitivity to nonphysiological divalent cations (barium, strontium, cobalt, etc) that has been demonstrated to be a genetically determined defect in the SHR (36–38).

The altered vascular sensitivity in hypertension may be regulated by the central nervous system, and the magnitude of the vascular change is demonstrably influenced by dietary factors. Several investigators (39–41) have shown that destruction of catecholamine-containing neurons in the central

nervous system reverses the increased contractile sensitivity to various agents and prevents the development of high blood pressure. It has also been shown that the dietary intake of certain minerals (calcium, magnesium), protein, and fatty acids alters the contractile function of blood vessels isolated from hypertensive animals, and that increased ingestion of these substances reduces blood pressure (40, 42–47).

CELL MEMBRANE RECEPTORS

The initial step leading to a change in the contractile properties of a vascular smooth muscle cell is the binding of the agonist to a receptor site located on the cell membrane. Altered sensitivity to vasoactive drugs in hypertension could derive from changes in the interaction between the agonist and the receptor sites on the vascular smooth muscle cell membrane. Relatively few studies have examined this possibility, but the major observations can be summarized as follows: (*a*) the number and affinity of alpha-adrenergic receptors on vascular cells is not sufficiently changed in hypertension to account for the increased sensitivity to alpha-adrenergic agonists (48–50); (*b*) there is a decrease in the number (but not in the affinity) of beta-adrenergic receptors in vascular smooth muscle cells in hypertension that parallels the inability of beta-adrenergic agonists to cause hypertensive arteries and veins to relax (51–53); and (*c*) the affinity of serotonergic receptors is not changed in hypertension, indicating that increased vascular sensitivity to the monoamine does not relate to this receptor property (54).

CALCIUM CHANNELS

Major regulators of the concentration of activator calcium in the vascular smooth muscle cell are the calcium channels in the plasma membrane through which calcium moves into the cell down its 10,000-fold concentration gradient. Van Breemen et al (55) have observed that this transmembrane movement of calcium into vascular smooth muscle obtained from hypertensive animals is greater than normal. The calcium channel through which calcium moves is a calcium binding protein, the function of which can be influenced by the amount of calcium bound to it. Johnson et al (56) observed this influence in a study of segments of porcine coronary artery. In their investigation, they rinsed this vascular smooth muscle several times in a calcium-free physiological salt solution and then depolarized it with 35 mM KCl to open the voltage-operated calcium channels. They then titrated the preparation with calcium, monitoring tension as a function of intracellular calcium concentration. Tension increased until the extracellular concentation reached 2.5 mM; then it declined. At 10 mM it had diminished to one third maximum; and at 30

mM the muscle was completely relaxed. They then were able to obtain a maximum contraction with 2×10^{-5} M histamine, which released calcium from internally sequestered stores. They thereby demonstrated that this relaxation was due to reduction of the concentration of cytosolic calcium in the muscle rather than to inactivation of the contractile protein. Johnson et al (56) conclude that calcium binds to a "calcium binding protein on the channel, producing structural changes in this calcium binding protein that result in channel blockade or inactivation."

The relevance of this channel to the cellular mechanism of hypertension was suggested to us in 1973 (57), when we observed that in hypertensive rats a higher concentration of calcium in vascular smooth muscle was required to produce relaxation than in normotensive rats. This was true for muscle from genetic, mineralocorticoid, and renal hypertensive rats. We interpreted this observation to indicate that there are fewer calcium binding sites on the plasma membrane of this muscle in hypertension, and that hence a higher concentration of calcium is required to produce membrane "stabilization" equivalent to that of the muscle in its normal state.

Recent observations have added support to the hypothesis that in hypertension there is a reduced amount of calcium bound to, and thus stabilizing the vascular smooth muscle membrane (Lamb, Moreland, & Webb, unpublished findings). Strips of rat aortae from SHR and WKY were treated for 5 minutes in a potassium- and calcium-free muscle bath. Addition of calcium (2mM) caused contraction of both strips. The rate of contraction was much more rapid in the aorta from the SHR than in that from the WKY. The responses were reduced to the same low level by treatment with nifedipine. In contrast, when they had been pretreated with 2mM EGTA to remove all calcium from the membrane, both strips contracted very rapidly when calcium was added to the bath. These observations were interpreted to indicate that the rate of contraction, or of calcium entry into the cell, was inversely related to the amount of calcium bound to the membrane. In the aorta from SHR, as compared to that from WKY, there is less calcium bound to the membrane in a manner that limits entrance of calcium through nifedipine-sensitive channels. Treatment with EGTA removes all the "stabilizing" calcium from the membrane with the result that calcium entry into the cell is very rapid. This potential-operated channel is also the protein binding site for the dihydropyridine compounds that, depending on the compound, may either block or activate this channel.

Our research has provided observations giving insight into the relationships between: (*a*) the action of a calcium entry blocker; (*b*) the effect of high calcium concentration (calcium stabilization); and (*c*) the hypertensive process (Webb, unpublished findings, 1984). We examined the effects of D600 on the ability of an elevated calcium concentration to relax strips of tail artery

from SHR and WKY. In methoxamine-contracted strips, the relaxant effect of elevated calcium concentrations was less in SHR than in WKY strips. D600 inhibited the calcium-induced relaxation of the arterial strips of both strains of rats; however, this inhibition occurred to a lesser degree for the strips from the SHR. We attributed the smaller ability of calcium to cause relaxation in SHR to a reduced number binding sites for calcium on the membrane, and a consequent decrease in susceptibility of the membrane to stabilization. The reduced inhibitory action of D600 suggests that this calcium entry blocker may be operating through the same reduced number of binding sites in the smooth muscle from the SHR.

Recently, studies have also been made of the effects of the dihydropyridine "calcium agonist" Bay K 8644 on vascular smooth muscle from, normotensive and hypertensive rats. This agonist opens rather than closes calcium channels. Asano et al (58) report that vascular smooth muscle from SHR is much more sensitive to Bay K 8644 than is that from WKY. Bruner & Webb (personal communication) have confirmed this difference, using strips of carotid artery from WKY and from stroke-prone SHR (SHRSP). In physiological salt solution (PSS) with normal potassium concentration, strips from SHRSP contracted in response to Bay K 8644, whereas those from WKY did not. When the concentration-response curve to Bay K 8644 was repeated in PSS containing 12 mM KCl, a small contraction developed in the WKY strip, and the magnitude of the response of the strip from the SHRSP was increased. When the KCl concentration was increased to 18 mM, both strips gave large and equal responses to Bay K 8644. It was concluded that in the resting vascular smooth muscle from SHRSP, more calcium channels are in a conformation that can be activated by Bay K 8644 than in that same muscle from WKY. This is a conformation that can be achieved by depolarization in vascular smooth muscle from either source.

These observations, together with those of the earlier studies on calcium stabilization of this channel, support the conclusion that in hypertension there is an abnormality in this potential-operated calcium channel.

Recently, Wright et al (59, 60) have extracted an interesting peptide from the red blood cells of SHR. The action of the peptide is relevant to the calcium channels of vascular smooth muscle, since it increases calcium uptake of aorta and potentiates the contractile response produced by depolarization with KCl. Smooth muscle from SHR is more sensitive to this peptide than is that from WKY. Single injections of minute amounts of the peptide either into the tail vein or into the 3rd ventricle of the brain produces arterial pressure elevations that reached a peak in 3–5 days.

Several investigators have used the patch clamp technique to characterize these channels in vascular smooth muscle (61–66). This muscle has two types of voltage operated calcium channels, one inactivated quickly, the other

slowly. More recently Rusch & Hermsmeyer (personal communication) have used this technique to compare cells from the azygous vein of neonatal SHR and WKY. They found that, although the total calcium current was the same in cells from these two sources, the relative proportion of "transient" and "sustained" currents was altered. Sustained current, thought to deliver the calcium that regulates contraction, comprised about two thirds of the current in SHR cells, whereas it comprised only one third of that in WKY cells. Calcium currents in the SHR cells became activated at lower electronegative potentials than did those from WKY. They believe that this difference occurring in neonatal individuals of these two strains would have the proper characteristic to explain a genetic component of increased peripheral resistance in the adults.

These calcium channels in the plasma membrane may be the most obvious regulators of cytoplasmic calcium concentration, yet this membrane has other important calcium regulatory systems. Of these, the following have been considered for the role they might play in hypertension: (*a*) phosphoinositide metabolism; (*b*) the sodium/calcium exchanger (Na/Ca); (*c*) sodium/potassium pump (Na/K ATPase); (*d*) the sodium/hydrogen exchanger (Na/H); and (*e*) the calcium efflux pump.

PHOSPHOINOSITIDE METABOLISM

The hydrolysis of membrane phosphoinositides in response to an agonist results in the liberation of two second messengers that may serve in a multifunctional transducing system (67–69). The two second messengers are: membrane-associated diacylglycerol and water-soluble inositol trisphosphate. Diacyglycerol activates protein kinase C, whereas inositol trisphosphate causes a release of calcium from intracellular membrane stores (67–69). Several studies suggest that both of these cellular processes accompany the contractile state in vascular smooth muscle (70–79).

In arteries from genetically hypertensive rats contractile sensitivity to protein kinase C activators (12-0-tetradecanoylphorbol-13-acetate and mezerein) is increased in comparison to arteries from normotensive rats (70). Furthermore, a selective inhibitor of protein kinase C [1-(5-isoquinolinesulfonyl)-2-methylpeperazine] blunts the enhanced sensitivity to serotonin in arteries from SHRSP (80). Since protein kinase C plays a role in the phosphorylation of several regulatory proteins (calcium-calmodulin, etc), it is possible that alterations in the activity of this enzyme contribute to the increased vascular sensitivity in hypertension.

Studies also indicate that the metabolism of inositol phosphates may be changed in arteries from hypertensive rats. Recent unpublished observations of Turla & Webb indicate that following treatment with serotonin release of

radiolabeled inositol phosphates in aortae from SHRSP is augmented in comparison to that in aortae from normotensive rats. These observations may explain the exaggerated phasic response to serotonin under calcium-free conditions in arteries from hypertensive rats (54). For it is known that inositol triphosphate causes a release of membrane-bound calcium (67–69), and that the initial phasic response in vascular smooth muscle is dependent upon activator calcium from an intracellular source.

SODIUM/CALCIUM EXCHANGER

The Na/Ca exchanger provides an attractive mechanistic explanation of the known relationships between dietary sodium intake and hypertension. A critical analysis of this exchanger in the squid axon (81) reveals that it is capable of operating in either direction across the membrane. Passive movement of either ion down its electrochemical gradient causes the movement of the other ion in the opposite direction. The transport process in vascular smooth muscle most investigated is the movement of sodium down its concentration gradient into the cell; this movement energizes the movement of calcium out of the cell against its metabolic gradient. It is possibly an important calcium efflux system. In accord with this possibility, experimental procedures that diminish the sodium concentration gradient decrease calcium efflux and hence increase contraction of this muscle. The decrease in sodium gradient is accomplished either by decreasing the sodium concentration of the physiological salt solution or by inhibiting the sodium pump, so that the intracellular concentration of this ion increases. Although the observed results are compatible with the operation of this membrane exchanger, there are alternative explanations. For instance, the aforementioned procedures could have potentiated the contraction by depolarizing the membrane, thereby opening potential operated calcium channels. In the assessments of Na/Ca exchangers, the breadth of the interpretations is somewhat narrowed by using plasma membrane preparations rather than isolated vessel segments or smooth muscle cells. Grover et al (82), using inside-out vesicles of plasma membrane preparations from uterine smooth muscle, observed that calcium was gained by sodium loaded vesicles when the sodium concentration of the medium was reduced. This happened even when the calcium was moved against a concentration gradient. However, the exchange was abolished when the membrane was made leaky by either the calcium ionophore, A23187, or the sodium ionophore, monensin. David-Dufilho et al (83) observed that the rate of sodium-dependent calcium efflux (Na/Ca exchange) in heart sarcolemmal vesicles from young rats was significantly greater in those from SHR than in those from WKY. Using electrophysiological technique, Hermsmeyer & Harder (84) were unable to find evidence of a Na/Ca exchanger in basilar and

caudal arteries from SHRSP and WKY. In contrast, Matlib et al (85) have observed that calcium uptake by sarcolemmal vesicles of mesenteric arteries from SHR and WKY is dependent upon sodium loading. The level of activity of the Na/Ca exchanger was greater in membrane vesicles from SHR, but the difference was not statistically significant.

The uncertainty about the role played by this exchanger in vascular smooth muscle function, and hence in the mechanism for hypertension, is emphasized in three recent reviews dealing with the Na/Ca exchanger in vascular smooth muscle (86–88). Brading & Lategan (86) conclude: "In spite of the strong possibility that blood vessels do possess a Na-Ca exchange mechanism, and the attractiveness of Na-Ca exchange as a mechanism which could link the known importance of Na with the increase in vascular resistance in hypertension, there is at present no direct evidence that it plays a significant role in the aetiology of hypertension."

SODIUM/POTASSIUM PUMP

The active membrane transport system for sodium and potassium has also been given much attention in regard to its connection with hypertension. The majority of studies that have evaluated this "sodium pump" in vascular smooth muscle have found that its activity is increased in hypertension (83, 89–93). However, other studies have reported that in hypertension a humoral substance in the plasma inhibits pump activity (94–99).

Different techniques have been employed to evaluate sodium pump activity in the vessel wall. David-Dufilho et al (83) measured ATP hydrolysis by plasma membrane vesicles from young SHR and WKY. They determined that the Na/K ATPase activity was twice as great in SHR as in WKY (22.2 + 2.6 vs. 11.3 = 1.6 μmol/h/mg), a finding that indicates an increased number of pump units. Other evaluations of pump activity have been more indirect. "Potassium relaxation" reflects the degree of membrane hyperpolarization caused by the electrogenic activity of the pump. Webb and his associates have observed that this relaxation is greater in vascular smooth muscle from hypertensive animals than in that from normotensive controls when studied in SHR (89), renal hypertensive rats (90) and DOCA hypertensive pigs (91). Hermsmeyer (92) has measured the membrane potential of the muscle in the rat tail artery in the presence (37°C) and absence (16°C) of the electrogenic pump. In the SHR this pump contributed 12 mV, whereas it contributed only 5 mV in smooth muscle from the WKY. Jones (93) found that the maximum efflux of sodium from rat aorta was increased by 40–50% following treatment with DOCA. These indirect observations have been interpreted as evidence of hyperactivity of Na/K ATPase that has been driven by a greater sodium leak into the cell.

Additional understanding of these relationships has been developed recently from studies of vascular smooth muscle cells after 9–11 passages in tissue culture. In these cells, the most consistent difference between those from SHR and WKY was the more rapid fluxes of ^{22}Na and ^{86}Rb observed in the SHR cell (100). The number of pump units, measured as ouabain binding sites, was significantly less in both SHR and WKY than in standard Wistar rats (101). Differences observed in these preparations must be primary genetic differences in the vascular smooth muscle, rather than the consequences of increased wall stress or secondary manifestations of circulating factors in vivo.

Sodium Pump Inhibitors

Whereas the studies already treated have been directed toward an understanding of the intrinsic activity of the Na/K ATPase in the vascular smooth muscle membrane, other investigators have considered that this activity may not be relevant to what happens in vivo. For a decade now, a strong case has been made that a humoral sodium pump inhibitor plays a role in the pathogenesis of hypertension. Several laboratories (94–99) have presented evidence that plasma from experimental animals or patients with hypertension contains a factor that inhibits sodium pump activity. This inhibitory action should increase total peripheral resistance by at least two means: (*a*) by decreasing the activity of the electrogenic pump, it will depolarize the plasma membrane and thereby open potential operated calcium channels; and/or (*b*) by decreasing sodium extrusion, intracellular sodium will accumulate, decreasing the transmembrane sodium gradient and thus decreasing the activity of the Na/Ca exchanger that is responsible for calcium extrusion from the cell. In addition to these actions that could produce hypertension, this factor is also considered to have a natriuretic effect by inhibiting sodium reabsorption from tubular urine. The factor is associated especially with hypertension in which there is volume expansion and sodium excess. In a review article that strongly supports the hypothesis that this factor plays a role in hypertension, Haddy (94) points out the end it probably serves: "Increased pressure and decreased reabsorption would be the best way to rid the body of the excess sodium and water."

Many different types of studies have supported the conclusion that in hypertension a factor in the plasma suppresses the activity of the sodium pump. For instance, Poston et al (95) found that sodium content of leukocytes from patients with essential hypertension to be about twice that of these cells from normotensive controls. The rate constant for the ouabain-sensitive sodium efflux was 50% greater in leukocytes from normotensive controls than in these cells from the patients with essential hypertension. However, when the normal leukocytes were incubated in serum from the hypertensive

patients, their sodium efflux rate constant was reduced to the same low level as in those from the hypertensive patients.

Hamlyn et al (96) monitored the hydrolysis of ATP by a partially purified Na/K ATPase from dog kidney. They demonstrated the inhibition of this enzyme by ouabain or vanadate, and found a highly significant correlation between the level of a plasma inhibitor of the enzyme and mean arterial blood pressure of normotensive and hypertensive individuals. Hamlyn has recently obtained an inhibitor of active sodium transport (STI) that increases in the plasma following volume expansion (personal communication). The inhibitor has been purified to apparent homogeneity and is a heat and acid stable polar compound of low molecular weight with chromatographic and mechanistic properties distinct from cardiac glycosides.

By radioimmunoassay, Gruber et al (97) determined the concentration of an endogenous plasma substance that reacted with goat antidigoxin antibody. They called the substance an endogenous digoxin-like factor and found that its plasma level paralleled the elevation of arterial pressure in Goldblatt hypertension in nonhuman primates.

Haddy & Pamnani (98) have amassed considerable evidence in support of their hypothesis that the "volume expanded" form of hypertension is caused by a circulating sodium pump inhibitor. They observed an inhibitory action of plasma from these animals on three assay systems: (*a*) ouabain-sensitive ^{86}Rb uptake in rat tail artery; (*b*) short-circuit current in toad bladder; and (*c*) membrane potential in rat tail artery.

Magargal & Overbeck (99) studied ouabain-sensitive ^{86}Rb uptake in primary cultured rat aortic smooth muscle cells. They found that plasma from rats with 1-kidney, 1-clip renal hypertension was significantly less able to stimulate this uptake than that from normotensive rats.

Songu-Mize et al (102) has presented evidence that the circulating pump inhibitor has its origin in the hypothalamus.

Boon et al (103) have recently presented evidence that raises serious questions as to this action of a sodium pump inhibitor in essential hypertension. They evaluated the sodium pump activity by the rate of rubidium clearance from the plasma and the rate of its appearance in the red blood cells. Rubidium was used as a marker for potassium. They compared these parameters in 22 untreated patients with essential hypertension with those in 22 carefully matched control subjects. This test revealed that patients receiving digoxin treatment and those with chronic renal failure had a reduction in Na/K ATPase activity, and that those with hypertension did not. Vo and Bohr have recently repeated a parallel evaluation of Na/K ATPase activity in DOCA hypertensive pigs (unpublished observation). Following the intravenous infusion of 5 μg/kg rubidium over a 100 min period, the rubidium level in the red

blood cells reached 40.3 + 4.5 μmole/l in the DOCA hypertensive pigs and only 23.8 + 2.4 μmol/l in the red blood cells of the control pigs. It is evident that if there is a circulating pump inhibitor in these animals, its action is masked by a more potent pump stimulation, presumably by an increase in intracellular sodium concentration (104).

We have presented evidence that in hyptertension, membrane permeability to sodium in vascular smooth muscle treated with ouabain increases. Intracellular sodium accumulates and the smooth muscle contracts, probably because the transmembrane gradient of sodium is diminished, lessening the calcium extrusion by means of the Na/Ca exchanger (88). In genetic (105), mineralocorticoid (106), and renal hypertensive rats (107), the aortic smooth muscle contracts to a greater extent than does that from their respective controls. We interpreted these observations as evidence that in hypertension the membrane is more permeable to sodium. This interpretation was supported by the observation that if normal aortic smooth muscle is treated with the sodium ionophore, monensin, its response is the same as that of such muscle from a hypertensive animal. On the other hand, the responsiveness of the aorta from the hypertensive animal could be reduced to normal by treatment with the sodium blocker amiloride.

These functional studies suggest that mineralocorticoid excess may cause a change in membrane permeability to sodium that can increase vascular smooth muscle responsiveness. A detailed analysis by Moura & Worcel (108) of this action on vascular smooth muscle membrane indicated that aldosterone produces an increase in sodium transport by three different mechanisms. They made their observations of the action of aldosterone on vascular smooth muscle both in vivo and in vitro. Two of the effects were delayed one or two hours and were completely blocked by actinomycin D. These effects, therefore, depended on stimulation by aldosterone of protein synthesis. One of the delayed effects was ouabain sensitive, which indicated that aldosterone had stimulated the production of the active sodium pump (membrane Na/K ATPase). The other delayed effect was ouabain insensitive, which demonstrated that aldosterone treatment had also produced a passive protein channel in the membrane. The third action by which aldosterone stimulated sodium efflux occurred within 15 min and was not inhibited by actinomycin D. Presumably, this stimulation represented a direct action of aldosterone on the plasma membrane. This effect, as well as the increase in sodium pump activity, occurred both in vivo and in vitro. However, stimulation of the delayed ouabain-insensitive system occurred only when aldosterone was administered in vivo.

This observation argues that the delayed increase in passive protein channels was secondary, resulting from the action of aldosterone on a control

system not in the blood vessel wall. Gomez-Sanchez (109) has observed that hypertension results from the administration of minute amounts of aldosterone into the lateral cerebral ventricles. This amount had no effect when administered systemically. This observation suggests that the factor responsible for the increase in ouabain insensitive sodium transport in vascular smooth muscle could be of central origin. If this is the case, this increase in passive sodium transport may be important in the development of hypertension. By increasing sodium influx, it may drive the sodium pump and thereby mask the action of a sodium pump inhibitor.

SODIUM/HYDROGEN EXCHANGER

Another membrane system that plays an important role in regulating sodium metabolism in the vascular smooth muscle cell is the Na/H exchanger. Little et al (110) present the following observations, which indicate that this exchanger accounts for most of the sodium influx in rat aortic smooth muscle cells in primary culture: (*a*) ethylisopropylaminoride, a specific inhibitor of Na/H exchange, blocks 80% of the sodium accumulation in ouabain treated cells; (*b*) acidification of the cell by exposure to nigericin or by incubation in a medium containing ammonium chloride increases ^{22}Na influx into the cell; this increase is prevented by treatment with ethylisopropylamiloride; and (*c*) sodium influx is greatly decreased by lowering the pH of buffer of the extracellular PSS. The increased sodium influx via the Na/H exchanger causes an increased activity of the Na/K ATPase, thereby maintaining intracellular sodium content approximately constant.

Cell swelling has also been used to measure the activity of the Na/H exchanger (111). When sodium propionate is substituted for sodium chloride in the PSS, the free propionate anion is in equilibrium with the proprionic acid. This acid is lipid soluble and therefore enters the cell. Intracellularly, it dissociates liberating H^+, which activates the Na/H exchanger. The continued presence of the weak acid leads to intracellular accumulation of sodium propionate. Uptake of osmotically obligated water then leads to cell swelling. Volume measurements are made by electronic cell sizing with a Coulter counter. No swelling occurs if potassium propionate is used instead of sodium propionate, since the Na/H exchanger does not exchange with potassium. In the presence of sodium proprionate, amiloride blocks the swelling.

Using this cell swelling evaluation of Na/H exchange, Feig et al (111) observed that the activity of this system was greater in thymocytes from SHR than in those from WKY or domestic Wistar rats. Livne et al (112) found that the exchanger was more active in blood platelets from patients with essential hypertension than in those from normotensive controls.

In a recent study, Muslin et al (113) presented evidence that supported the importance of the Na/H exchanger in hypertension. They noted that amiloride, an antagonist of Na/H exchange, decreases blood pressure in SHR but not in WKY. They found that the Na/H exchange in vascular smooth muscle cell culture was twice as great in cells from SHR as in those from WKY. They also observed a greater acidification in the SHR cells in response to angiotensin II. The acidification was accompanied by a much greater increase in intracellular calcium concentration. They concluded that the enhanced Na/H exchange may contribute to the increased vascular contraction in SHR.

CALCIUM EXTRUSION PUMP

This plasma membrane system for regulating intracellular calcium concentration is universal. Schatzmann (114) emphasized the importance of this function when he asserted that "living matter is distinct from the rest of the universe in not putting up with the prevailing Ca^{2+} concentration." He has characterized this calcium extrusion pump as it is found in the red blood cell, where it is most easily studied. It is a calcium and magnesium requiring ATPase of approximately 140,000 molecular weight. It is stimulated by calmodulin and inhibited by vanadate. Its characteristics clearly differentiate it from the pump that sequesters calcium in the sarcoplasmic reticulum.

Several investigators have studied this calcium extrusion pump in vascular smooth muscle.

Popescu et al (115, 116) have demonstrated the presence of this calcium and magnesium-dependent ATPase in scarolemmal membranes of pig coronary artery. They observed that nitroglycerin stimulates the calcium extrusion pump and concluded that this is the mechanism by which this agent produces coronary dilatation. Other investigators have shown that this activity occurs in the bovine aorta (117) and pig coronary artery (118). Evidence has also been presented indicating that some of the vasodilator activity of nitrendipine results from its stimulation of the calcium extrusion pump (119).

Kwan et al (120) compared the ATP-dependent calcium accumulation into inside-out sarcolemmal vesicles from mesenteric arteries of normotensive and hypertensive rats. This measure of active calcium extrusion was reduced in both SHR and mineralocorticoid-induced hypertension.

Postnov and Orlov (121), in support of their hypothesis that the membrane abnormality in hypertension is generalized to all tissues, present evidence that the calcium uptake by plasma membrane vesicles from rat brain is 40% less in SHR than WKY.

OVERVIEW

Figure 1 indicates that specific initiating factors can be used experimentally to produce hypertension by increasing total peripheral resistance. This increase in vascular resistance is accompanied by an increase in vascular smooth muscle sensitivity. This review surveys experimental evidence bearing on possible mechanisms responsible for the increase in sensitivity. The mechanisms have the form of plasma membrane systems involved in the regulation of intracellular ionized calcium concentration. Evidence can be found that in hypertension, each of the many regulatory systems changes in such a way that it could be responsible for the increase in cellular calcium. The problem confronting the field at the present time is to determine which of the membrane changes is primary and therefore plays the most important role in increasing vascular smooth muscle sensitivity in hypertension. The bias of the authors of the present review is that in hypertension there is a generalized defect or deficit in the calcium binding protein of the plasma membrane, and that this defect is responsible for a lack of membrane stability.

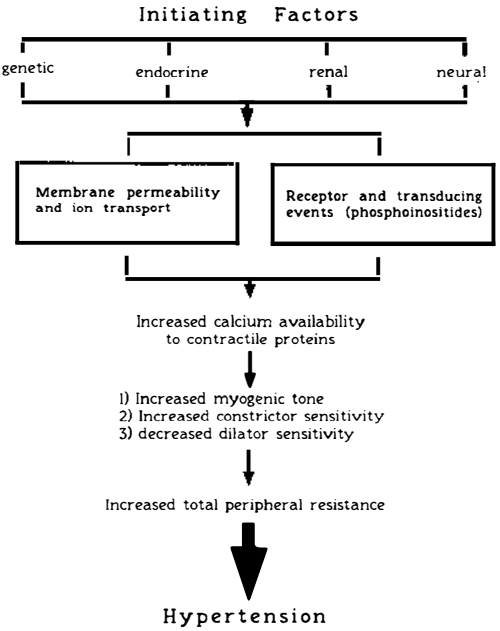

Figure 1 Involvement of the plasma membrane of the vascular smooth muscle cell in hypertension.

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