Membrane Mechanisms in Arterial Hypertension

DAVID R. HARDER, PH.D., AND KENT HERMSMEYER, PH.D.

SUMMARY The purpose of this review is to focus on alterations in vascular muscle membrane potentials ($E_m$), ionic permeabilities, and ionic transport systems which may either contribute to or be a consequence of the hypertensive state. Three models of hypertension are discussed: 1) deoxycorticosterone-salt (DOCA-salt)-induced hypertension; 2) low-renin (presumably volume expanded) renal hypertension (LRRH); and 3) the spontaneously hypertensive rat (SHR) of the Okamoto-Aoki Kyoto-Wistar strain and its normotensive genetic control (WKY). The importance of studying all possible mechanisms of increased contraction in vascular smooth muscle is stressed.

(Hypertension 5: 404-408, 1983)

KEY WORDS • DOCA-salt hypertension • low-renin renal hypertension • spontaneously hypertensive rat • vascular smooth muscle • ionic permeability • transport systems • blood pressure regulation • membrane potential

Although neural and humoral factors influence both central and peripheral sites to regulate arterial pressure (Brody,1 Abboud2), the final common pathway for the control of vascular reactivity, and ultimately peripheral vascular resistance, lies at the level of the vascular smooth muscle cell. Thus, knowledge of the membrane processes responsible for vascular muscle cell activation is crucial in understanding the complete scope of blood pressure regulation. Furthermore, it is important to recognize the differences found in different blood vessels (Hermsmeyer,3-4 Harder et al.5). For valid conclusions to be drawn, the same vessel should be compared in hypertensive and normotensive animals. Mechanisms worked out in a particular blood vessel can be rigorously applied only to that particular vessel, unless others of interest have also been vigorously investigated. For a comprehensive theory of altered vascular muscle membrane function, the same measurements should be carried out on each vessel of interest. As data from different blood vessels are only available for some of the alterations found, it is difficult to draw more than qualified conclusions about the generality of differences.

This overview will encompass the following concepts regarding the vascular muscle cell membrane and its control over force development in the hypertensive vs normotensive state: differences between in vivo and in vitro membrane potential ($E_m$), including the effects of an intact sympathetic innervation; altered ionic permeabilities in hypertensive vascular smooth muscle and the concomitant effect on vascular reactivity; and alterations in energy-dependent electrogenic ion transport as a function of humoral factors, innervation, or intrinsic muscle properties in vascular muscle cells from animals with hypertension of three different origins.

In Vivo vs In Vitro Membrane Potentials in Vascular Muscle Cells of Spontaneously Hypertensive Rats

Extensive literature has accumulated demonstrating that sympathetic control of the vasculature in spontaneously hypertensive rats (SHR) is altered at many levels (Okamoto et al.,6 Yamori,7 Dietz et al.,8 Folkow et al.,9 Takahashi and Bunag,10 Abboud1). Furthermore, alterations in release and uptake of norepinephrine (NE) may produce effects similar to increased neural input and may contribute to the maintenance of the hypertensive state (Abboud1).
It is only in the SHR that both in vivo and in vitro data are available because of the great difficulty in measuring $E_m$, especially in vivo. The $E_m$ of small mesenteric veins of SHR compared to WKY differ markedly when measured in vivo, but not when measured in vitro (Harder et al.\cite{11}). The difference in $E_m$ between SHR and WKY in vivo is abolished if neural input is blocked by tetrodotoxin (Harder et al.\cite{11}) or if $\alpha$-adrenergic receptors are blocked by phenoxybenzamine (Willems et al.\cite{12}). These differences are summarized in Table 1. Such findings strongly suggest that the adrenergic excitation of the vascular muscle cell membrane is markedly elevated in mesenteric veins of SHR. It is unlikely that such differences in $E_m$ between SHR and WKY mesenteric veins are due to increased stretch (preload) since the in vivo pressure within mesenteric veins of SHR is only several millimeters of mercury higher than that recorded in WKY (unpublished observations). Differences in $E_m$ are also absent in isolated portal veins (Hermesmeyer and Walton\cite{14}).

Such in vivo studies use the $E_m$ of vascular muscle cells as a sensitive indicator that sympathetic stimulation of individual vascular muscle cells is stronger in SHR. The usefulness of this technique lies in the fact that the membrane response is the "final common pathway" indicator of neurotransmitter access to vascular muscle, combining the following influences: impulse activity in sympathetic nerve fibers, amount of NE released per impulse, uptake and inactivation of NE, and vessel wall thickness and connective tissue influencing NE diffusion. Recording of intracellular potential demonstrates that there is elevated sympathetically influenced on mesenteric veins of SHR (Harder et al.\cite{11}, Willems et al.\cite{12}). Furthermore, by using specific blockers of $\alpha$- and $\beta$-receptors, it is possible to demonstrate an endogenous hyperpolarizing influence (possibly epinephrine) which appears to be masked in SHR mesenteric veins by the predominance of $\alpha$ adrenergic response (Willems et al.\cite{12}).

It is interesting to note that, even after neural blockade via tetrodotoxin, the $E_m$ measured in the same vessel in vivo is about $6\,\text{mV}$ less negative than when in vitro in both SHR and WKY when the vessels are studied under identical conditions in an organ bath (Harder et al.\cite{11}) (table 1). This finding might demonstrate that blood-borne substances exert a degree of control over vascular muscle cells, if the stretch and other isolation procedures can be taken to have minimal effect.\cite{11}

When the vascular muscle cell $E_m$ of SHR and WKY are measured in vitro, no significant difference in the resting value can be found, even though conditions are optimized for each vessel. No difference in resting $E_m$ between SHR and WKY was found in mesenteric veins (Harder et al.)\cite{11} caudal artery (Hermesmeyer\cite{14}), portal vein (Hermesmeyer and Walton\cite{14}), or middle cerebral artery (Harder et al.)\cite{14}. In both caudal and middle cerebral arteries, however, we have found alterations in membrane electrical properties that suggest altered electrogenic ion transport, which are unmasked only after inhibition of ion transport.

### Membrane Control Over Force Development: Abnormalities in Hypertensive States

#### $E_m$ and Tension

Membrane control over force development in vascular muscle is defined by corresponding changes in $E_m$ and mechanical events, but all of the variables allowing the relationship to be changed are unclear (Hermesmeyer et al.\cite{15}). However, there exists substantial literature demonstrating that a high degree of the activation state at the level of the plasma membrane is associated with contraction (Harder\cite{19}). Briefly, depolarization of vascular muscle cells increases the influx of $\text{Ca}^{2+}$ with resultant force development (Johansson and Somlyo\cite{15}) and agonist stimulation depolarizes $E_m$ and thus increases ionic conductances, including $\text{Ca}^{2+}$, through voltage sensitive channels (Haeusler,\cite{20} Harder and Sperelakis\cite{19}). The level of $E_m$ prior to agonist stimulation largely determines the sensitivity to that agonist (Hermesmeyer\cite{19}) because the distributions and conductances of ions across the vascular muscle plasma membrane are influenced by $E_m$. Ion gradients and conductances thus determine and are influenced by $E_m$ in a complex interaction. $E_m$ is a major control of the mechanical properties of the blood vessel within a given vascular bed, thereby ultimately controlling peripheral vascular resistance in the intact animal (Hermesmeyer,\cite{14} Hermesmeyer et al.,\cite{15} Harder\cite{19}). Although there is no evidence that the relationship between $E_m$ and contraction is shifted in hypertensives vs normoten-

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*Each $E_m$ value is the mean ± SE calculated from n impalements, with external suffusion solution (PSS) as zero reference, a = no. of animals used to obtain the total number of impalements. WKY = Wistar-Kyoto rats; SHR = spontaneously hypertensive rats; $E_m$ = transmembrane potential; PSS = physiological saline solution; TTX = tetrodotoxin.

†Magnitude of in situ $E_m$ in SHR is significantly greater than corresponding in situ value. (From Harder et al., see ref. 11, reproduced by permission of the American Physiological Society.)

**Magnitude of in vitro $E_m$ in WKY or SHR is significantly greater than corresponding in situ value, (From Harder et al., see ref. 11, reproduced by permission of the American Physiological Society.)
sives, ionic mechanisms leading to increased contraction through changes in $E_m$ under certain conditions have been identified. These include alterations in ion gradients, passive ionic conductances, and energy-dependent ion transport, any one of which can alter the response to an agonist as will be considered below.

The previous section alluded to the involvement of the sympathetic nervous system in increased peripheral resistance. The following sections will focus on altered ionic permeabilities and transport systems in steroid, renal, and genetic models of hypertension.

**Deoxycorticosterone-Salt (DOCA-Salt) Hypertension**

The early history describing electrolyte abnormalities and proposing low salt diets as a treatment in hypertension has been reviewed by Tobian, Bohr and others who have since demonstrated several properties likely to be explained by altered ionic permeabilities and/or transports. For example, femoral artery strips from DOCA hypertensive rats develop spontaneous mechanical oscillations upon elevation of $[Ca]_o$, a phenomenon observed much less frequently in normotensive controls. Alterations in the ionic permeabilities and turnover of aortic arterial muscle of DOCA hypertensive animals were also found by Jones and Hart, who demonstrated increases in $K^+$ and $Cl^-$ fluxes across plasma membranes. Similarly, Jones observed increased $Na^+$ flux in aortas of DOCA hypertensive rats.

There is no agreement on whether the altered responsiveness of arteries from DOCA hypertensive animals are the result of an elevated cell volume or primary changes in the ionic composition (Cox; Cohen et al.). What does appear to be clear, however, is that changes in ionic permeabilities and/or transmembrane gradients can change both sensitivity and reactivity to an agonist (Cox; Cohen et al.). In the DOCA-salt hypertensive animal model, it has been suggested that the increase in vascular reactivity may initiate the increase in mean arterial blood pressure (Berecek and Bohr). If so, the change in ion permeabilities may be transient because the work of Hermsmeyer and colleagues demonstrated no difference in resting $E_m$ or mechanical response to norepinephrine in caudal arteries of DOCA-salt hypertensive rats. However, Webb found increased relaxation in vitro on return to $K^+$ in pig femoral and tail arteries of DOCA hypertensives. The DOCA-salt model may well be dependent on a circulating ouabain-like factor that would not be detected in vitro (Pamnani et al.). Friedman suggested that aldosterone-stimulated $Na^+$ transport is greater in rat caudal arteries in DOCA-salt hypertension, and that perhaps mineralocorticoid activity contributes to other humoral factors to cause a net increase in intracellular $Na^+$. Vasopressin also appears necessary to the development of hypertension in the DOCA-salt rat (Berecek et al.).

**Renal Hypertension (LRRH)**

Vascular muscle has a sizeable electrogenic component to its resting $E_m$ contributed by ion transport, probably through Na-K ATPase. This electrogenic component can range from 8 to 20 mV depending upon the artery (Hermsmeyer; Harder). The Na-K pump suppression appears to increase responsiveness in vascular smooth muscle cells of animals with experimental low renin, presumably volume-expanded hypertension (Overbeck). The evidence for reduced Na-K pump activity includes decreased $K^+$-induced vasodilation and decreased ouabain-sensitive $Rb^+$ uptake by hypertensive blood vessels (Overbeck et al.; Pamnani et al.), and decreased Na-K ATPase activity in cardiac microsomes (Clough et al.). The immediate result of electrogenic Na-K pump inhibition by an endogenous, ouabain-like substance on vascular muscle would be membrane depolarization. Indeed, such a mechanism has been supported in hypertensive arterial muscle (Pamnani et al.). The resulting depolarization of $E_m$ would lead to a smaller threshold to reach the contraction range of $E_m$ in response to vasoconstrictor agents in renal hypertensive animals, unless other ionic mechanisms were also altered.

**Spontaneously Hypertensive Rats (SHR)**

Several investigators have demonstrated that aortas from SHR contract to the divalent cations $Sr^{++}$, $Co^{++}$, and $Mn^{++}$, while their genetically normotensive controls do not (Shibata et al.; Bohr; Greenberg and Bohr, Noon et al., Webb and Bohr), suggesting that SHR arterial muscle cells are "leaky" to divalent cations. However, muscular arteries do not contract in $Sr^{++}$, $Co^{++}$, or $Mn^{++}$ (Hermsmeyer and Walton). Why aortas would be contracted by foreign cations is not established, but it probably is the consequence of an increased cell permeability to $Ca^{++}$, thereby augmenting the hypertensive state.

On the other hand, even smaller muscular arteries of SHR show greater contraction in high $Ca^{++}$ solutions than WKY (Webb and Bohr). This increased sensitivity to divalent cations in vascular muscle cells of hypertensives has also been attributed to the inability of structures within the cell to bind and/or extrude intracellular $Ca^{++}$ (Webb and Bhalla; Shibata et al.; Noon et al.; Moore et al.; Kwan et al.). There is also indirect evidence that $Ca^{++}$ permeability is greater in arteries from essential hypertensive individuals (Hultén et al.) as well as SHR (Lederballe Pedersen et al.). It has been well documented that arterial muscle cells from the SHR have an elevated ion transport activity (Jones; Hermsmeyer; Hermsmeyer and Walton; Webb and Bohr; Harder et al.; Friedman). This enhanced pump activity in SHR caudal arterial muscle membranes increases the electrogenic component to the resting $E_m$ by 5 to 7 mV over those of WKY (Hermsmeyer). It has been hypothesized that an elevated electrogenic component partially compensates for a reduced $K^+$ equilibrium potential ($E_K$) in the hypertensive vascular muscle plasma membrane (Hermsmeyer; Harder). A reduced $E_K$ in arterial muscle would increase the sensitivity to vasoactive agents which mediate their action through membrane depolar-
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ization because it would bring the diffusion potential closer to mechanical threshold. In moderate to high concentrations of NE, the SHR cells would be more depolarized and contracted (Hermsmeyer1-4).

Although an enhanced electrogenic component to the arterial muscle $E_m$ in SHR appears to be a property of arteries from several different vascular beds, a reduced $E_m$ does not. Middle cerebral arterial muscle cells treated with ouabain to block electrogenic ion transport are depolarized more in SHR than in WKY rats, suggesting an enhanced electrogenic ion transport similar to other arteries; however, when $E_m vs \log [K]$ curves are used to determine the $E_m$, there is no difference (Harder et al.5). The difference between the $E_m$ even with electrogenic pumping inhibited in SHR and WKY cerebral arteries is due to a difference in $E_m-[K]$ slope, suggesting a difference in Na$^+$/K$^+$ permeability. Perhaps a related phenomenon is the consistent finding of spontaneous electrical and mechanical activity in cerebral arteries from SHR (Harder et al.5).

One influence that does not appear important in SHR is that of the humoral factors that seem to be involved in DOCA-salt and renal forms of hypertension. The first evidence is that, unlike in SHR, the $E_m$ of DOCA-salt rats in solutions at 16°C or in 0 K$^+$ solutions were not different from controls. This suggests that the SHR have an altered ion transport mechanism that persists even in vitro (after the humoral factors would have been washed out). The second bit of evidence is that the SHR form of hypertension is the only one so far tested that is not prevented by the lesion of the anteroventral region of third ventricle (AV3V) developed by Brody and colleagues (Brody1). It thus appears that, in SHR, neural factors and the trophic influence of the sympathetic nervous system are more important factors. The trophic influence is suggested because it was possible to demonstrate by cross-inner-vation experiments that the altered membrane NE sensitivity and $E_m$ electrogenesis follow the innervation, rather than the source animal for the caudal arteries (Abel and Hermsmeyer45). There does not appear to be evidence that the SHR is dependent upon a humoral ouabain-like factor.

Conclusions

Consideration of evidence that has been proposed to give cellular mechanisms leading to altered NE sensitivity and contraction in DOCA-salt, renal, and SHR forms of hypertension suggests that different mechanisms are involved, and are worthy of separate exploration. Although ion transport systems seem implicated in each of the forms of hypertension, the precise forms of ion transport that are altered, and the dependence of the alterations on neuronal, humoral, or inherent vascular muscle properties, might be quite different. The most important point in studying all of these types of hypertension is to identify and understand thoroughly each altered mechanism that can contribute to increased contraction. It has long been understood that hypertension is a multifaceted disease. Comparison of these differently induced forms of hypertension only emphasizes the importance of finding all possible mechanisms leading to abnormally high tension development by vascular muscle cells.

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Hypertension. 1983;5:404-408
doi: 10.1161/01.HYP.5.4.404

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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