MINIREVIEW

Ion Channels in Vascular Smooth Muscle: Alterations in Essential Hypertension (44286)

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Abstract. **Essential hypertension is characterized by a near normal cardiac output but an increase in total peripheral resistance. In turn, total peripheral resistance is controlled directly by the diameter of the small arteries and arterioles like those in the kidney. The dynamic regulation of renal vessel diameter is governed by the contractile state of the vascular smooth muscle cells that line the vessel walls. This review addresses the role of a number of different ion channels to initiate and maintain the contractile state of the vascular smooth muscle cells in hypertension and the potential prevention of hypertension through gene therapy. These specific channels include Ca^{2+}, K_{Ca}, K_v, and Cl^- channels. In hypertension, it has been reported that increased activity of Ca^{2+} channels and decreased activity of K_v channels are responsible for the increased contractile tone and resting membrane potential observed in dissociated vascular smooth muscle cells from the spontaneously hypertensive rat. In contrast, increased activity of K_{Ca} channels in vascular smooth muscle cells of the SHR has been hypothesized to dampen or brake the activity of Ca^{2+} and K_v channels. Finally, recent evidence suggests that introducing angiotensin II type-1 receptor antisense into prehypertensive rat pups prevents the onset of pathophysiological alterations observed in hypertension including K^+ channel alterations. These results suggest that gene therapy may be a useful pharmacological and physiological tool to combat hypertension.**
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The diagnostic measure of hypertension is an elevation in blood pressure that is determined by cardiac output and total peripheral resistance (TPR). Essential hypertension is characterized by a near normal cardiac output but an increase in total peripheral resistance (1). TPR in turn is controlled directly by vessel diameter particularly that of the small arteries and arterioles. The dynamic regulation of vessel diameter is governed by the contractile state of the vascular smooth muscle cells that line the vessel walls. Smooth muscle contraction, like that of striated muscle, is controlled by increases in intracellular Ca^{2+} concentration. Calcium binds to calmodulin at four distinct "EF-hand" sites, inducing a conformational change in the binding protein (2). The Ca^{2+}-calmodulin complex is then able to activate myosin light chain kinase that transfers a phosphate from ATP to a serine at position 19 on the 20-kD light chain of myosin (3). This allows the contractile myofilaments (actin and myosin) to interact and initiate cross-bridge cycling. As long as intracellular ATP is present and the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) remains elevated over basal levels, contraction of the smooth muscle is maintained.

In vivo, vascular smooth muscle exists in a state of partial contraction. In this state, termed myogenic tone, levels of $[Ca^{2+}]_i$ exceed that observed in the relaxed state (4, 5). These levels of $[Ca^{2+}]_i$ are maintained by the balance between calcium influx and extrusion (for review see Refs. (6–8)). A stimulatory rise in $[Ca^{2+}]_i$ may lead to further

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contraction of the vascular smooth muscle. This process is termed excitation-contraction coupling of which there are two recognized mechanisms. The first, electromechanical coupling, operates through changes in the vascular smooth muscle cell's membrane potential. The second, pharmacomechanical coupling, operates through agonist stimulation of second messenger signaling pathways to increase $[Ca^{2+}]_i$ and does not necessarily involve prior membrane depolarization. These mechanisms are not independent of each other (3); however, both require an increase in myoplasmic free Ca^{2+}. The two pathways leading to this increase in $[Ca^{2+}]_i$ are: 1) release from intracellular stores; and 2) influx from the extracellular space. The release of Ca^{2+} from the sarcoplasmic reticulum is associated with a transient contraction. Whereas, maintained contraction is dependent on Ca^{2+} influx across the sarcolemma. Although, it has been demonstrated that agonist-stimulated $[Ca^{2+}]_i$ levels and contraction are altered in hypertension, it is the maintained contraction that is important in the long-term regulation of arterial pressure.

Two major routes exist for the stimulated entry of extracellular Ca^{2+} in vascular smooth muscle; voltage-gated Ca^{2+} channels and receptor operated channels (ROCs). Other mechanisms of Ca^{2+} entry have been identified (i.e., nonselective cation channels, calcium release–activated calcium channels (I_{crac}), and stretch-activated channels). However, the nature of their role in the control of vascular tone is not clearly established, and it has yet to be determined if these mechanisms are truly separate and unique pathways of Ca^{2+} entry. For example, it has been proposed that Ca^{2+} entry through ROCs may not be distinct from that of voltage-gated Ca^{2+} channels or that the main function of ROCs is to shift the membrane potential into the range where voltage-gated Ca^{2+} channels have an increased conductance (8–10). Nevertheless, Ca^{2+} influx through voltage-gated Ca^{2+} channels has been established as important in the control of vascular tone.

Since Ca^{2+} channels in vascular smooth muscle are strongly voltage-dependent, their function is closely linked to changes in membrane potential. Plasmalemma membrane potential is determined by the permeability of several ions. Whereas, Na^+ flux is controlled mainly by pumps and exchangers (Na^+-Ca^{2+} exchanger or Na^+/K^+-ATPase), a variety of ion channels permit the passage of Ca^{2+}, K^+, and Cl^- across the membrane. This review will focus on the nature of these channels in vascular smooth muscle and how changes in their current may contribute to membrane potential alterations in hypertension.

Voltage-Gated Calcium Channels

To date, two subtypes of voltage-dependent Ca^{2+} channels have been identified in vascular smooth muscle: L- and T-type (7). L-type Ca^{2+} channels in vascular smooth muscle are activated by relatively strong depolarizations. The channels inactivate slowly and incompletely and are blocked by dihydropyridines. In contrast, T-type Ca^{2+} channels are activated at more negative potentials. These channels exhibit rapid, voltage-dependent inactivation and are insensitive to dihydropyridines. L-type Ca^{2+} channels appear to be the major Ca^{2+}-influx pathway in vascular smooth muscle. Entry through this channel is important in the control of resting tone (11). The influx through T-type Ca^{2+} channels is small, transient, and likely to be inactivated at rest. Therefore, the remaining discussion will focus on L-type Ca^{2+} channels.

In smooth muscle, L-type Ca^{2+} channels are composed of multiple subunits: α_1, α_2/δ, β (12). The α_1-subunit is the pore-forming central component necessary for ion selectivity, voltage sensing, activation, and inactivation (13). The α_1 also contains binding sites for the organic (dihydropyridines, phenylalkylamines, and benzothiazepines) and inorganic (Cd^{2+}, Ni^{2+}) Ca^{2+} channel blockers (14). The highly conserved α_2/δ subunit is a dimer that is linked together with a disulfide bond and may be involved in membrane anchoring. The β subunit appears to be a modulatory subunit that shifts the voltage dependence of activation and inactivation to more negative membrane potentials (15).

The dependence of the L-type Ca^{2+} channels on membrane potential is important in the control of vascular tone (for review see Ref. 9). The overlapping nature of the L-type Ca^{2+} channel activation and inactivation curves predicts a small sustained Ca^{2+} influx through this channel. This has been termed the Ca^{2+} window current and its reported values fall within the range of *in vivo* measured smooth muscle membrane potential. Therefore, it may account for the steady-state levels of $[Ca^{2+}]_i$ necessary for maintained vascular tone (16, 17). L-type Ca^{2+}-channel open probability (P_o) increased sharply with membrane depolarization, changing approximately e-fold for 6–8 mV (9). This steep voltage dependence allows the L-type Ca^{2+} channels to respond to small graded changes in membrane potential. Therefore, L-type Ca^{2+} channels provide a regulatory mechanism for the interdependence of Ca^{2+} influx, membrane potential, and vessel diameter (9, 18, 19).

The molecular components responsible for L-type Ca^{2+} channels consist of five polypeptide subunits: α_1, α_2-γ, β, and δ (13). The α_1 subunit is required for formation of a functional channel. Tissue specific isoforms are believed to contribute to L-type Ca^{2+} channel diversity. Three different genes (class C, D, and S) encoding for L-type Ca^{2+} channel α_1 subunits have been identified (20). Complementary DNA encoding for the α_1 subunit (class C) has been cloned from rat aorta, and evidence suggests that this sequence may exist as an alternatively spliced isoform of the cardiac α_1 (21).

Smooth Muscle K^+ Channels

Whereas depolarization of vascular smooth muscle relies on the activation of Ca^{2+} conductances, membrane repolarization is dependent on the opening of plasmalemmal K^+ channels. The opening of K^+ channels causes plasma membrane hyperpolarization and inhibition of voltage-dependent Ca^{2+} entry. Given the negative equilibrium potential for K^+, vascular smooth-muscle K^+ channels not only

dampen membrane depolarization but also play a prominent role in the maintenance of resting membrane potential and resulting myogenic tone. Using the patch clamp technique, at least three types of calcium-activated K^+ (K_{Ca}) channels, two types of voltage-activated, calcium-insensitive potassium (K_v) channels, and an ATP-dependent potassium (K_{ATP}) channel have been linked to repolarization in vascular smooth muscle. In addition, an inwardly rectifying K^+ channel (K_{ir}) may play a role in regulation of membrane potential (for review see Refs. 22–24).

Ca^{2+}-Activated K^+ Channels. Several types of K_{Ca} channels that differ in unitary conductance (small, intermediate, or large conductance) and pharmacology have been identified in various nonvascular tissues. In vascular smooth muscle, the current most often described is a large-conductance ($\geq$200pS)-, Ca^{2+}-, time-, and voltage-dependent K^+ current. It has been identified in a number of vascular beds including: rabbit pulmonary artery (25), portal vein (26), ear artery (27), canine coronary (28), renal artery (29), rat renal resistance vessels (30), and cultured aortic cells (31). Membrane depolarization (~2.7-fold increase/12–14 mV depolarization) as well as physiologically relevant increases in $[Ca^{2+}]_i$ increase the P_o of K_{Ca} channels (32). Pharmacologically, external tetraethylammonium (TEA), charybdotoxin (ChTx), iberiotoxin (IbTx), EGTA, and Ni^{2+} inhibit channel activity. The molecular components responsible for the large conductance K_{Ca} current have been described. The initial cloning of this class of K_{Ca} channels was from the fruit fly *Drosophila,* and the gene has been termed slowpoke (*d*slow) (33). Vogalis *et al.* recently cloned and expressed cDNAs encoding the α- and β-subunits of the *d*slow gene from canine smooth muscle (34).

The physiological role of large conductance K_{Ca} channels in vascular smooth muscle is well understood (for review see Refs. 22, 32). The membrane depolarization and elevation of $[Ca^{2+}]_i$ associated with vasoconstriction will cause activation of K_{Ca} channels. This activation is under partial regulatory control by small, concentrated bursts of Ca^{2+} release (Ca^{2+} sparks) from the sarcoplasmic reticulum (35). The resulting activation of these channels causes membrane hyperpolarization and relaxation of arterial tone (36). Finally, K_{Ca} channels are regulated by a number of endogenous vasoactive agents. Vasoconstrictors such as angiotensin II, thromboxane A_2, and muscarinic agonists have been linked to inhibition of K_{Ca} channels (37–39) whereas activators of protein kinase A (PKA) and G (PKG) appear to increase channel activity (36). This further suggests a physiological role for K_{Ca} channels in the regulation of vascular tone.

Recently, a second type of K_{Ca} channel was found to coexist with the large conductance K_{Ca} channel in vascular smooth muscle from rat renal arterioles (40). This channel had a unitary conductance of 68 pS and was selectively blocked by apamin (50 n*M*). An apamin-sensitive K^+ current has also been identified in rabbit mesenteric arteries (41). However, the contribution and role of this K_{Ca} channel needs to be elucidated.

Voltage-Dependent K^+ Channels. Voltage-dependent K^+ (K_v) channels comprise the largest and most diverse class of ion channels. This class can be subdivided into three major families based on their response to membrane voltage, pharmacology, and molecular identity. These include: 1) the delayed rectifier K^+ currents; 2) the A-type K^+ currents; and 3) the inward rectifier K^+ currents, all of which have been identified and characterized in vascular smooth muscle. As the name implies, the inward rectifier K^+ channels possess unique rectification properties that allow the channels to play a separate role in vascular smooth muscle. Therefore, for the purpose of this discussion, the K_{ir} channels will be discussed separately.

K_v channels appear to be ubiquitously expressed. They have been identified, and the current characterized in a variety of vascular beds including: portal vein (26, 42), coronary artery (43), cerebral artery (44), renal vasculature (29, 30), pulmonary artery (25), and the mesenteric vasculature (45). The outwardly rectifying current carried by delayed rectifier K^+ channels turns on with a brief delay following membrane depolarization to potentials positive to ~–40 mV. This current exhibits slow C-type inactivation that involves a conformational change in the channel pore (46). In contrast, the A-type K^+ current activates steeply with voltage between –90 mV—60 mV and then decays spontaneously and rapidly. Inactivation of the A-type, sometimes referred to as the transient K^+, current has been termed N-type because it involves a pore-blocking particle tethered to the N-terminus of the channel (47). Both currents are insensitive to removal of extracellular Ca^{2+} and are selectively inhibited by low concentrations of 4-aminopyridine (4-AP).

The issue of multiple K_v channel subtypes within vascular smooth muscle is complex and may not be easily classified into the above mentioned subfamilies. Biophysical and pharmacological evidence suggests the existence of multiple components of K_v current within a single tissue. Also the existence of different K^+ channel distributions among various vascular beds or changes along an arterial tree have also been shown (48, 49). On a molecular level, K_v channels are traditionally represented in four gene families (K_v1-K_v4) based on homology to those initially cloned from the *Drosophila* (*Shaker, Shab, Shaw,* and *ShaI* genes). However, new families of gene products are rapidly being identified. Within each family, alternative splicing and gene duplication give rise to many channel subtypes (46). In addition, the possibility of heterotetramer formation in smooth muscle may also increase diversity and further complicate the identification of such components (50). To our knowledge only $K_v1.2$ (51), $K_v1.5$ (52), K_v 1.1 (53), and $K_v2.2$ (Horowitz B, personal communication) have been cloned and characterized in smooth muscle. However in vascular smooth muscle, the molecular components for only $K_v1.5$ and $K_v2.2$ have been demonstrated using Western blot analysis.

Voltage dependent K^+ channels have several physiological roles in vascular smooth muscle. One important role is to dampen excitation by providing a mechanism of membrane hyperpolarization. Nelson *et al.* calculated that due to the high input resistance of vascular smooth muscle cells, only a small number of K_v channels need to be open to limit membrane depolarization and facilitate vasorelaxation (9). A second important role is the contribution of K_v channels to vascular smooth muscle resting membrane potential. The steep relationship between steady state P_o and membrane voltage, as determined from the mean activation and inactivation curves, suggest the presence of a significant sustainable K_v current at rest (22). In addition, pharmacological blockade of K_v channels leads to membrane depolarization in current clamped, isolated, vascular smooth muscle cells as well as constriction of intact vessels (54–57). In our laboratory, we observe similar results in rat renal resistance arterioles, shown in Figure 1. The bath application of both Ang II and 4-AP causes membrane depolarization (~20–25 mV) of dissociated smooth muscle cells and contraction of an intact resistance artery from WKY rats. This provides additional evidence for the role of K_v channels in the regulation of vascular smooth muscle membrane potential. Finally, it has been proposed that K_v channels are regulated by a number of endogenous vasoactive agents. Vasoconstrictors such as angiotensin II, histamine, and hypoxia have been associated with inhibition of K_v channels (57–59).

Inward Rectifier K^+ Channels. Inward rectifier K^+ (K_{ir}) current has been identified in several vascular beds including cerebral (60), coronary (61), and mesenteric vasculature (62). Interestingly, these channels appear to be localized in small-resistance-size vessels (i.e., <200 μm). The channels display a time-independent rectification that is opposite to the driving force established by the K^+ ion gradient. They open with steep voltage dependence upon hyperpolarization; however, they pass a small sustained outward current at potentials positive to the K^+ equilibrium potential (E_K). The channel is also very sensitive to changes in extracellular K^+ ($[K^+]_o$) possessing the ability to shift its voltage dependence of gating with changes in E_K. Micromolar concentrations of extracellular Ba^{2+} cause a voltage dependent block of K_{ir}. Inhibitors of other K^+ channels (i.e., 4-AP, TEA, glibenclimide) appear to have no effect on K_{ir}. However, millimollar concentration of Cs^+ also appear to block channel activity.

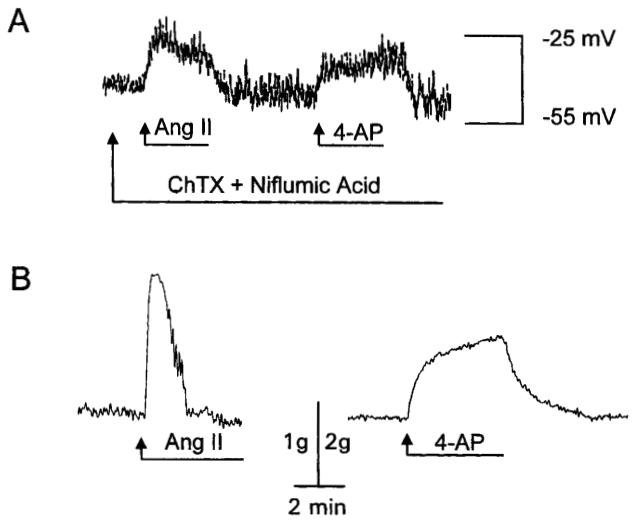

Figure 1. Effect of Ang II and 4-AP on electrical and contractile properties of renal resistance arterioles. (A) Current clamp experiment on isolated single vascular smooth muscle cell in the presence of ChTX (100 n*M*) and niflumic (100 μ*M*) acid. The bath application of both Ang II (100 n*M*) and 4-AP (3 m*M*) caused membrane depolarization. (B) Tension measurements of intact interlobar artery. Ang II (100 n*M*) caused a transient contraction of the vessel whereas 4-AP (3 m*M*) caused a sustained contraction that relaxed after washout.

An inward rectifier K^+ channel is yet to be cloned from smooth muscle. However, it is expected to share a similar structure to that of cloned K_{ir} channels from other tissues. K_{ir} is believed to assemble as a tetramere with each subunit having two hydrophobic segments very similar to the S5, S6, and H5 segments that comprise the pore region of the Shaker-like K^+ channels.

Similar to the molecular identity, the physiological role of K_{ir} in vascular smooth muscle is not well defined. As mentioned above, K_{ir} channels do conduct a small amount of outward current at potentials positive to E_K. Therefore, in the absence of depolarizing factors they may contribute to the maintenance of resting membrane potential. Low concentrations of Ba^{2+}, selective for K_{ir}, have been shown to depolarize vascular smooth muscle from small cerebral and coronary arteries (23, 63, 64). However, this is in the absence of transmural pressure, which may be significant (65, 66). The unique sensitivity of K_{ir} to changes in extracellular K^+ may suggest another physiological role for this channel. Several pathophysiological conditions, such as hypoxia or ischemia, result in the elevation of $[K^+]_o$. K_{ir} is one mechanism of vasorelaxation by which vascular smooth muscle cells may respond to this increase (66–68). However, a second mechanism, activation of the Na^+/K^+ pump, may underlie the vasodilatory response. Nevertheless, the physiological role and molecular identity of K_{ir} in vascular smooth muscle remains unclear.

ATP-Sensitive K^+ Channels. A potassium current that is voltage independent and inhibited by the noncovalent binding of ATP has been identified in several vascular beds, including the pulmonary (69), coronary (70, 71), and mesenteric vasculature (72, 73). Whereas all of the currents from these tissues are blocked by glibenclimide, the nature of the single channel conductance that underlies K_{ATP} is unclear. The literature describes two populations of channels of varying unitary conductance (large: 130–260 pS; small: 10–30 pS) depending on the preparation, vascular bed, recording method, and Ca^{2+} sensitivity (for review see Ref. 74). K_{ATP} channels are composed of at least two subunits believed to co-assemble as heterotetramers. Two subunits similar to the truncated Shaker-like K_{ir} proteins combine with the sulfonylurea receptor (Sur) to form functional

K_{ATP} channels (75). These subunits have recently been cloned from smooth muscle (Horowitz B, personal communication), and their heterologous expression will certainly provide further insight into their unitary conductance and cellular regulation.

Although ATP inhibits channel activity, adenosine diphosphate (ADP) increases K_{ATP} current, at least in the portal vein (76–78). In addition adenosine has been shown to activate K_{ATP} and cause vasodilation (70). Also, activation of K_{ATP}, *via* a cAMP/PKA, second-messenger pathway contributes to the calcitonin gene-related (CGRP) mediated relaxation of rabbit mesenteric arteries (79, 80). Endothelial factors such as prostacyclin and nitric oxide (NO) also appear to activate K_{ATP} channels (41). The NO-dependent activation of K_{ATP}, like that of CGRP, appears to be transduced *via* a second messenger pathway, but in this case involving cGMP (81, 82). In contrast to the agents that cause arterial relaxation, a number of endogenous vasoconstricting agents including Ang II (83), endothelin (84), and vasopressin (85), have been shown to inhibit K_{ATP}. However, the mechanism of this inhibition is yet to be elucidated.

The functional role of K_{ATP} in vascular smooth muscle is diverse. It has been suggested that K_{ATP} plays an important role in reactive hyperemia, endotoxic shock, and metabolic regulation of blood flow. Taking into account the large number of endogenous vasoactive agents that target K_{ATP}, this channel must be important in the regulation of vascular tone. However, this appears to be dependent on the vascular bed. For example, glibenclimide constricts arteries from the coronary and mesenteric vasculature but does not affect those from the renal, cerebral, or pulmonary vascular beds (for review see Ref. 22). In addition, a number of K^+-channel-opening drugs appear to cause activation of K_{ATP}. These drugs, including minoxidile, dizoxide, pinacidil, and cromakalim, cause direct vasodilation and have been used as antihypertensive agents.

Chloride Channels. Two major types of chloride-selective conductances have been identified in vascular smooth muscle. One is a voltage-gated chloride current that has been reported only in cultured rat embryonic aortic cells (86). The chloride conductance most often reported in vascular smooth muscle is that of a Ca^{2+}-dependent Cl^- ($I_{Cl(Ca)}$) current with a small unitary conductance of ~2–3 pS (for review see Ref. 87). Patch clamp recordings first identified $I_{Cl(Ca)}$ as one component of current underlying the norepinephrine-evoked depolarization of rat anaccoxycygeus muscle cells (84). Since then $I_{Cl(Ca)}$ has been identified in a number of vascular tissues, including portal vein (88–90), ear artery (91), mesenteric artery (92), pulmonary artery (90), and rat renal resistance arteries (93).

$I_{Cl(Ca)}$ is strongly Ca^{2+} dependent with this relationship varying between tissue and species. One study in rat portal vein reports the half-maximal activation of $I_{Cl(Ca)}$ at ~365 n*M* (94). However, it appears that $I_{Cl(Ca)}$ is not as sensitive to $[Ca^{2+}]_i$ as K_{Ca} (87). Also, it has been suggested that unlike K_{Ca}, activation of $I_{Cl(Ca)}$ may require both Ca^{2+} and a second unknown substance. This claim is based on the rapid rundown of $I_{Cl(Ca)}$ in whole cell and excised patch recordings. This decrease in channel activity was prevented using nystatin-perforated patches (95). In addition $I_{Cl(Ca)}$ is weakly voltage dependent with an e-fold increase in P_o for an ~100 mV change in membrane potential (compare this to Ca^{2+} and K_v current) (87).

In order to understand the functional role of $I_{Cl(Ca)}$, it is necessary to examine the Cl^- ion distribution in vascular smooth muscle cells. The presence of secondary, active Cl^- transport mechanisms (i.e., Cl^-/HCO_3^- exchanger and the Na^+-K^+-$2Cl^-$ co-transporter) within the plasmalemma of smooth muscle cells results in the accumulation of intracellular chloride above that expected from passive distribution (96–98). Therefore, the Cl^- equilibrium potential is approximately −20 mV. Since the resting membrane potential of vascular smooth muscle cells is more negative than this (approximately 50 mV), activation of Cl^- channels will lead to a net Cl^- efflux resulting in membrane depolarization. $I_{Cl(Ca)}$ is activated in vascular smooth muscle by a number of vasoactive agents, including norepinephrine (84, 88), ATP (99, 100), endothelin (92, 93), histamine (101), and vasopressin (102). This activation appears to be mediated by the stimulatory release of Ca^{2+} from intracellular stores (87). The resulting membrane depolarization may recruit more voltage-dependent calcium channels to open producing contraction. It has also been proposed that $I_{Cl(Ca)}$ may be activated by Ca^{2+} sparks from the SR. This current, termed spontaneous transient inward currents (STICS), has been recorded in several vascular tissues (103, 104). Finally, $I_{Cl(Ca)}$ may play a role in the regulation of resting membrane potential. It is hypothesized that membrane permeability to Cl^- may explain why the resting membrane potential of vascular smooth muscle is positive to E_K. Recently, Nelson *et al.* (105) has suggested that a niflumic acid (niflumic acid is a selective inhibitor of $I_{Cl(Ca)}$)-insensitive Cl^- current may contribute to myogenic tone in rat cerebral arteries. However much work is needed to identify specific Cl^- channels contributing to vascular tone. Nonetheless, the most probable role for $I_{Cl(Ca)}$ is its contribution to excitatory, agonist-induced vasoconstriction (87, 106).

Recently, a third type of Cl^- channel has been identified in vascular smooth muscle. Yamazaki *et al.* (107) report the functional and molecular expression of a volume-regulated chloride channel in canine pulmonary and renal vascular smooth muscle cells. This channel identified as the CLC-3 chloride channel may represent a unique mechanism of membrane potential modulation and control of myogenic tone.

Ion Channel Alterations in Hypertension

As mentioned previously, essential hypertension involves a gradual and sustained increase in total peripheral resistance. These changes in vessel diameter must involve alterations in the contractile state of vascular smooth

muscle. In both human and animal models of essential hypertension, it has been demonstrated that the resting vascular tone is elevated, and the contractile response to a number of physiological stimuli is enhanced compared with normotensive controls. Both of these changes will contribute to the increased blood-flow resistance. A number of cellular mechanisms in vascular smooth muscle may contribute to these changes in hypertension (Fig. 2). The focus of this discussion will be alterations in plasma membrane ion channels.

To date, the majority of fluorescence and electrophysiological studies of vascular changes in essential hypertensin have been performed on *large conduit vessels.* Conflicting results regarding changes in contractility (108), resting membrane potential (30, 109–112), basal $[Ca^{2+}]_i$ (30, 113, 114), and the onset of ion channel alterations (115) in conduit vessels from models of essential hypertension may be due to differences in the vascular bed studied. Mulvaney *et al.* have suggested that the increased contractility observed in vascular beds from hypertensive models must reside in the resistance vessels (116). Moreover, the large conduit vessels play a less significant role in setting peripheral vascular resistance than do smaller resistance vessels (117). Studies of clinically relevant resistance vessels (i.e., renal or cerebral vasculature) may provide further insight into the role of ion channel alterations in the development and maintenance of essential hypertension.

In the literature, a multitude of evidence suggests that altered ionic permeabilities exist in vascular smooth muscle in hypertension (1, 118). Using the patch clamp technique and vascular smooth muscle cells dissociated from *conduit arteries,* it has been demonstrated that there is an increased L-type Ca^{2+} current density in vascular smooth muscle cells from the spontaneously hypertensive rat (SHR), a model of human essential hypertension (119–123). In addition, it has also been shown that the K_{Ca} current density in the aorta from hypertensive rats is larger than the control (110). This alteration was observed in single-channel recordings of the K_{Ca} current from excised aortic membrane patches in which a greater Ca^{2+} sensitivity of the SHR K_{Ca} was demonstrated (114). This increase in K_{Ca} may also be due to an upregulation of channel expression resulting from a change in some post-transcriptional event (124). Finally, Ohya *et al.* (125) have reported an impaired action of levocromakalim on K_{ATP} channels in smooth muscle cells from the SHR. These data suggest that alterations in K_{ATP} function or density may exist in hypertension.

Using dissociated cells from physiologically relevant, renal resistance arterioles (Fig. 3), we have investigated changes in K^+ currents and membrane potential in genetic and nongenetic models of hypertension. The Wistar-Kyoto (WKY) cells are elongated and spindle shaped, and they reversibly contract to the bath application of Ang II (100 n*M*). The SHR cells are also spindle shaped yet are contracted compared to the WKY cells. However, there is no difference in the cell surface area as measured by cell capacitance (30). SHR cells also contract to the application of Ang II, but to a greater extent than the WKY cells. These data may reflect an elevated basal and agonist-stimulated $[Ca^{2+}]_i$ that we reported previously (30). The patch clamp technique was used to examine the differences in WKY, Sprague-Dawley (SD), SHR, and deoxycorticosterone ac-

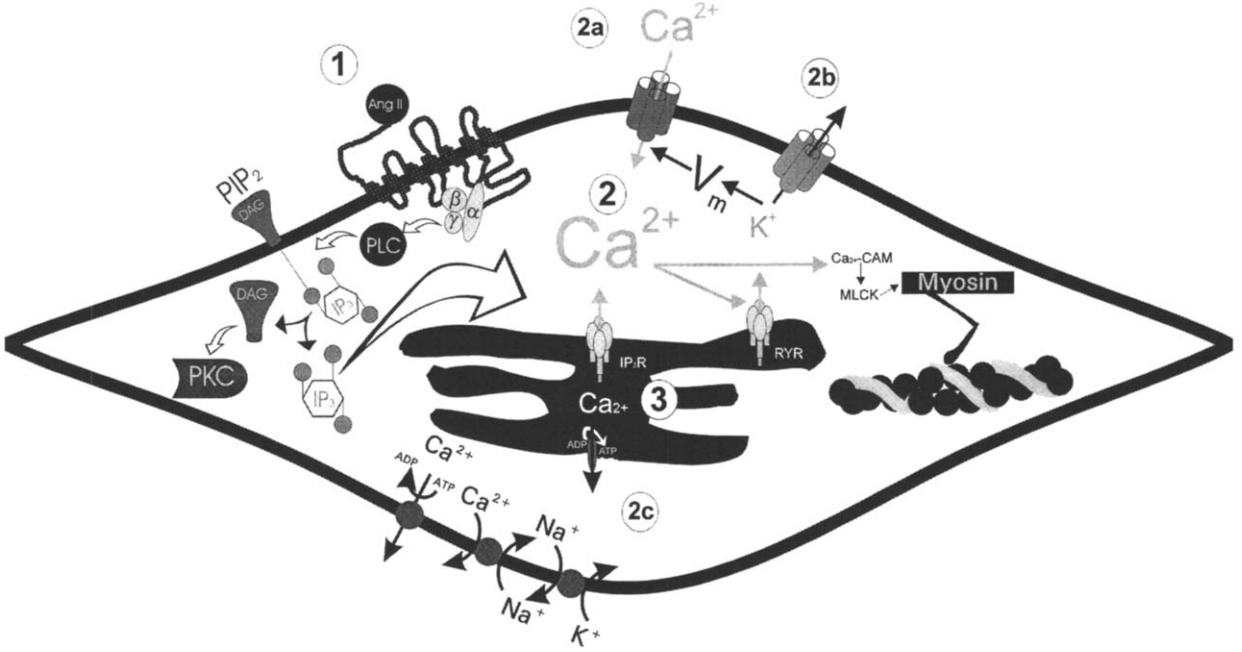

Figure 2. Cellular mechanisms in vascular smooth muscle cells that may contribute to contractile alterations in essential hypertension: (1) Increased sensitivity to circulating hormones such as angiotensin II. (2) Increased levels of myoplasmic free Ca^{2+}. This may result from (2a) increased Ca^{2+} current, (2b) decreased K^+ current, (2c) altered activity of pumps and exchangers in the SL or SR, (3) increased Ca^{2+} loading of the SR, or (4) increased Ca^{2+} sensitivity of the contractile myofilaments.

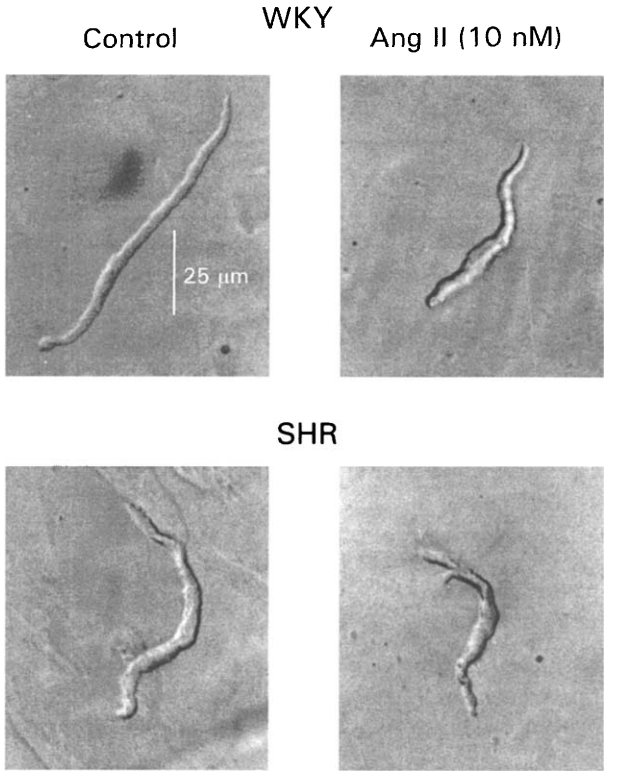

Figure 3. Renal resistance artery vascular smooth muscle cells of the WKY and SHR. Representative micrographs of cells from a WKY and SHR rat renal arteriole. Both cells contract to the bath application of Ang II (10 n*M*).

etate hypertensive (DOCA) single cells of the renal resistance arteries (30). In current clamp experiments, SHR and DOCA hypertensive cells were approximately 20 mV more depolarized than the control. In voltage clamp experiments with 4-AP and niflumic acid present to inhibit K_v and $I_{Cl(Ca)}$ currents, SHR and DOCA hypertensive K_{Ca} current was significantly larger and activated at more negative potentials than the control. Conversely, with ChTx and niflumic acid present to inhibit K_{Ca} and $I_{Cl(Ca)}$ currents, SHR and DOCA hypertensive K_v was significantly smaller than the control. Basal and angiotensin II-stimulated peak $[Ca^{2+}]_i$ was greater in the SHR and DOCA hypertensive cells when compared to the control. Finally, we have also observed an augmented SHR and DOCA Ca^{2+} current in these cells (Figs. 4 and 5). These results suggest that membrane potential and the activity of Ca^{2+} and K^+ channels are altered in hypertensive rat renal interlobar arteries and may play a role in the regulation of renal blood flow under physiological and pathophysiological conditions. These results also suggest that these alterations are attributable to the hypertensive state and not to genetic differences (SHR versus DOCA).

Reversal of Ion Channel and Pathophysiological Alterations Associated with Hypertension

Hypertension produces pathophysiological changes that are often responsible for the mortality associated with the disease. However, it is unclear if normalizing blood pressure with conventional therapy is effective in reversing the pathophysiological damage. The duration and initiation of treatment, site of administration, and agent used all appear to influence the reversal of the pathophysiological alterations associated with hypertension. A major question regarding ion channel alterations in vascular smooth muscle cells and the development of essential hypertension is one of cause versus effect. That is, do the observed changes in ionic current underlie the development of hypertension, or do they simply follow changes in blood pressure? The relationship between blood pressure changes and ion channel alterations has been addressed in a few studies. Rusch and Runnells (126) showed that normalization of high blood pressure in SHR using ramipril, an angiotensin-converting

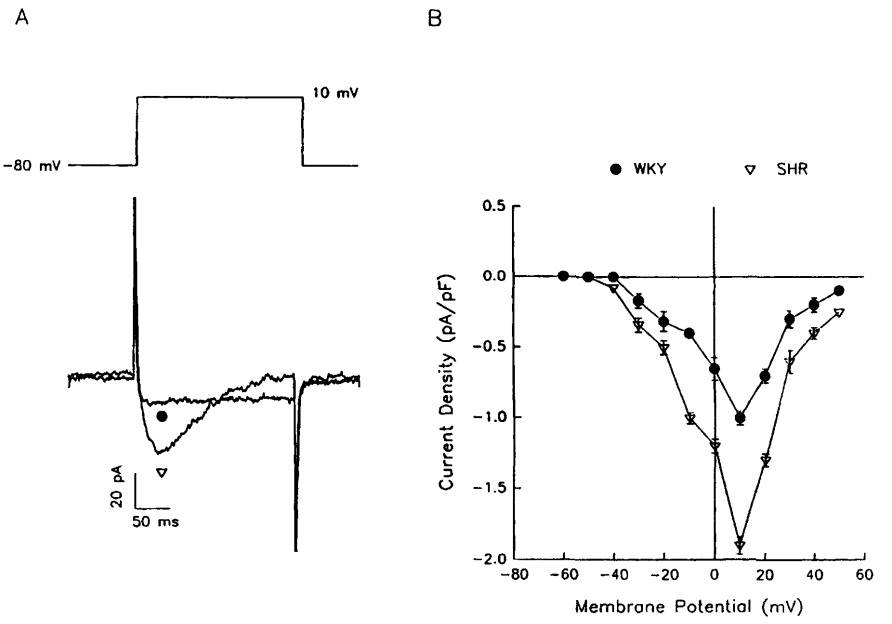

Figure 4. Ca^{2+} currents in WKY and SHR smooth muscle cells. Ca^{2+} (2.5 m*M*) currents were measured using the patch clamp technique on vascular smooth muscle cells dissociated from rat renal resistance arterioles. (A) Representative Ca^{2+} current recorded from WKY and SHR cells during a step depolarization from a holding potential of -80 mV to 0 mV. (B) Current-voltage relationship for WKY and SHR cells normalized to cell capacitance (n = 8).

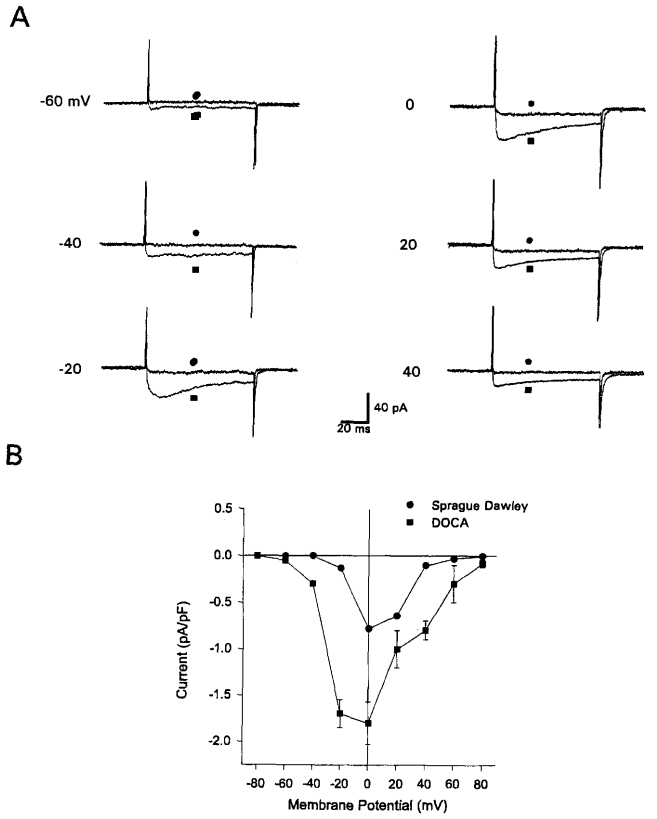

Figure 5. Ca^{2+} currents in SD and DOCA smooth muscle cells. Ca^{2+} (2.5 m*M*) currents were measured using the patch clamp technique on vascular smooth muscle cells dissociated from rat renal resistance arterioles. (A) Representative Ca^{2+} current recorded from SD and DOCA cells during a step depolarization from a holding potential of −80 mV to 0 mV. (B) Current-voltage relationship for WKY and SHR cells normalized to cell capacitance (n = 8).

enzyme inhibitor (ACE), reversed the increase in K_{ca} observed in the aorta (126). In addition, Ohya *et al.* (125) reported that the impaired action of levcromacalim on K_{ATP} channels in SHR was corrected with pharmacological antihypertensive treatment including hydralazine, Ca^{2+} channel blockers, and ACE inhibitors.

We have previously established that retrovirally mediated delivery of angiotensin II type-1 receptor antisense (AT_1R-AS) attenuates the development of hypertension in the SHR (56). Our objective was to determine if this attenuation of hypertension is associated with prevention of other pathophysiological changes induced by the hypertensive state (127). Intracardiac delivery of AT_1R-AS in neonates prevented the development of hypertension in the SHR for at least 120 days. Contractile experiments demonstrated an impaired endothelium-dependent vascular relaxation (ACh, Fig. 6) and an enhanced contractile response to vasoactive agents (KCl: Fig. 7; and phenylephrine: Fig. 8) in the SHR renal vasculature. In addition, the voltage-dependent K^+ (K_v) current density, which is believed to contribute to smooth muscle resting membrane potential and basal tone, was decreased in renal resistance artery cells of the SHR. AT_1R-AS treatment prevented each of these renal vascular alterations. Finally, AT_1R-AS delivery pre-

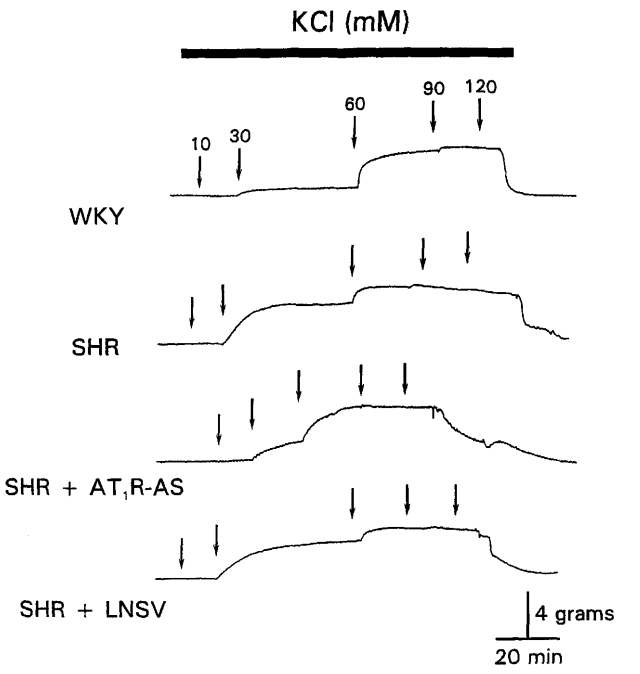

Figure 6. Prevention of the alterations in the KCl concentration response relationship of rat renal artery in hypertension with AT_1R-AS. The KCl concentration-response relationship was shifted to the left in the SHR and SHR + LNSV when compared to the WKY. There was no significant difference in the concentration-response relationship for SHR + AT_1R-AS when compared to the WKY. Representative of at least six different experiments.

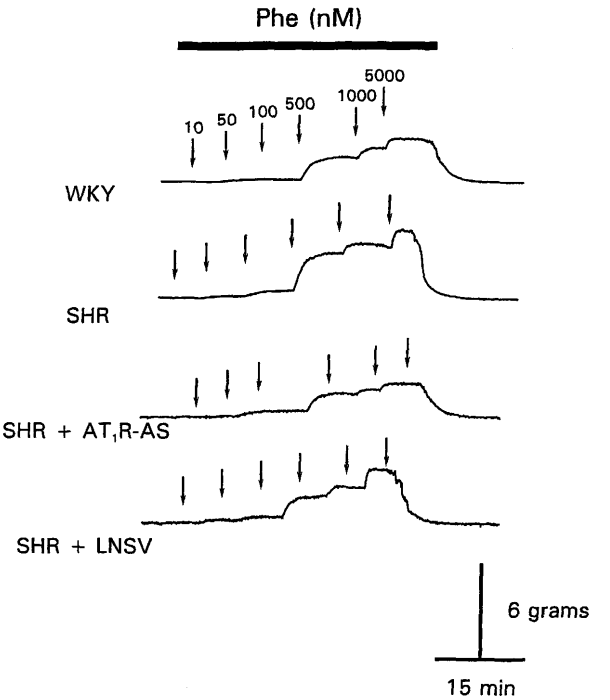

Figure 7. Prevention of the alterations in the phenylephrine concentration-response relationship of rat renal artery in hypertension with AT_1R-AS. The phenylephrine (Phe) concentration-response relationship was shifted to the left in the SHR and SHR + LNSV when compared to the WKY. There was no significant difference in the concentration-response relationship for SHR + AT_1R-AS when compared to the WKY. Representative of at least six different experiments.

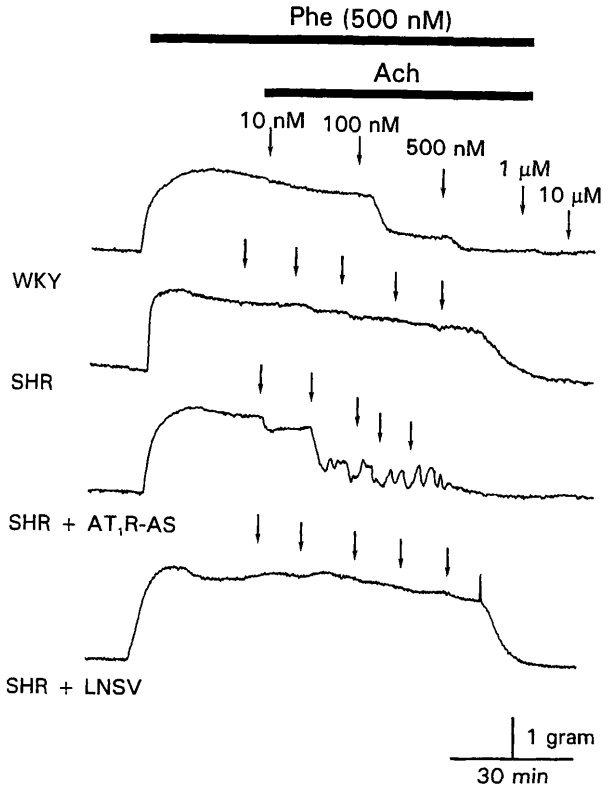

Figure 8. Prevention of the alterations in the acetylcholine concentration-response relationship of rat renal artery in hypertension with AT_1R-AS. The acetylcholine (Ach) concentration-response relationship was shifted to the right. The maximum relaxation is smaller in the SHR and SHR + LNSV when compared to the WKY. There was no significant difference in the concentration-response relationship for SHR + AT_1R-AS when compared to the WKY. Representative of at least six different experiments.

vented the pathological alterations observed in the SHR myocardium, including left ventricular hypertrophy, multifocal fibrosis, and perivascular fibrosis. These observations demonstrate that viral-mediated delivery of AT_1R-AS attenuates the development of hypertension on a long-term basis and that this is associated with prevention of pathophysiological changes in the SHR. These findings also suggest that the pathophysiological alterations are secondary to changes in blood pressure or distal to an angiotensin-triggered event.

Future Perspectives

Alterations in excitation-contraction coupling and ion channel function occur in the SHR model of essential hypertension. The use of gene therapy with antisense to the renin-angiotensin system pathway, given to pre-hypertensive SHR pups, has been proven successful in the prevention of the above pathophysiological alterations observed in hypertension. Future studies in our laboratory will try to elucidate the role of ion channel alterations in the etiology and maintenance of essential hypertension. We will also continue our investigation of the use of antisense gene therapy for the remission of high blood pressure and the reversal of ion channel alterations in hypertension. These studies will focus on the treatment of adult SHR with established essential hypertension. Finally, we will begin to mate some of our SHR AT_1R-AS virus treated pups with one another to see if the prevention of hypertension is passed on to their offspring, and investigate if the retrovirus permanently modifies the SHR genome.

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