Vascular smooth muscle cell membrane depolarization after NOS inhibition hypertension

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Bratz, Ian N., Ricardo Falcon, L. Donald Partridge, and Nancy L. Kanagy. Vascular smooth muscle cell membrane depolarization after NOS inhibition hypertension. Am J Physiol Heart Circ Physiol 282: H1648–H1655, 2002; 10.1152/ajpheart.00824.2001.—Nitric oxide (NO) synthase (NOS) inhibition with N^o-nitro-l-arginine (l-NNA) produces l-NNA hypertensive rats (LHR), which exhibit increased sensitivity to voltage-dependent Ca^{2+} channel-mediated vascular smooth muscle tone. We hypothesized that enhanced contractile responsiveness after NOS inhibition is mediated by depolarization of membrane potential (E_m) through attenuated K^+ channel conductance. E_m measurements demonstrated that LHR vascular smooth muscle cells (VSMCs) are depolarized in open, nonpressurized (−44.5 ± 1.0 mV in control vs. −36.8 ± 0.8 mV in LHR) and pressurized mesenteric artery segments (−41.8 ± 1.0 mV in control vs. −32.6 ± 1.4 mV in LHR). Endothelium removal or exogenous l-NNA depolarized control VSMCs but not LHR VSMCs. Superfused l-arginine hyperpolarized VSMCs from both the control and LHR groups and reversed l-NNA-induced depolarization (−44.5 ± 1.0 mV vs. −45.8 ± 2.1 mV). A Ca^{2+}-activated K^+ channel agonist, NS-1619 (10 μM), hyperpolarized both groups of arteries to a similar extent (from −50.8 ± 1.0 to −62.5 ± 1.2 mV in control and from −43.7 ± 1.1 to −55.6 ± 1.2 mV in LHR), although E_m was still different in the presence of NS-1619. In addition, superfused iberiotoxin (50 nM) depolarized both groups similarly. Increasing the extracellular K^+ concentration from 1.2 to 45 mM depolarized E_m, as predicted by the Goldman-Hodgkin-Katz equation. These data support the hypothesis that loss of NO activation of K^+ channels contributes to VSMC depolarization in l-NNA-induced hypertension without a change in the number of functional large conductance Ca^{2+}-activated K^+ channels.

NS-1619; vascular smooth muscle cells; potassium channels; nitric oxide synthase

VASCULAR SMOOTH MUSCLE TONE, an important determinant of peripheral vascular resistance and blood pressure, is largely determined by vascular smooth muscle cell (VSMC) intracellular Ca^{2+} concentration. Vascular tone is actively increased by a reduction of the absolute magnitude of VSMC membrane potential (E_m). Kuriyama et al. (21) reported a range of E_m for VSMCs of −45 to −60 mV. In VSMCs, large conductance Ca^{2+}-activated K^+ (BKCa) channels, voltage-operated K^+ channels, and ATP-sensitive K^+ (K ATP) channels are the major contributors to potassium conductance (gK), and activation of any of these channels leads to VSMC hyperpolarization with a consequent closure of voltage-dependent Ca^{2+} channels (VDCCs), decreased Ca^{2+} entry, and vasodilation. Thus K^+ channel activity is an essential determinant of vascular tone and vessel diameter.

There is increasing evidence that changes in expression and permeability of ion channels contribute to the vascular pathology of hypertension by causing depolarization of VSMC E_m. Indeed, VSMC depolarizations of 5 to 20 mV have been demonstrated in several models of hypertension (12, 24, 25, 34, 38). The mechanisms responsible for VSMC depolarization in hypertension have not been precisely defined, although there is evidence for a role of K^+ -Ca^{2+}, and Cl^- channels. For example, studies of Ca^{2+} channel density have shown unchanged (40), increased (20, 26, 32), or decreased expression (13) in various models of hypertension. Liu et al. (23) demonstrated an increase in expression of BKCa channels in aortas isolated from spontaneously hypertensive rats. In contrast, Silva et al. (38) demonstrated that triethylammonium induced less depolarization in spontaneously hypertensive rat aortas than in normotensive Wistar-Kyoto rat aortas, suggesting a change in K^+ channel activity or expression. Lamb et al. (22) reported that increased Cl^- efflux may contribute to the depolarization. These findings suggest that a combination of altered channel expression and ion conductances may account for VSMC depolarization in hypertension.

Endothelial cells release several vasoactive factors, including nitric oxide (NO), which can alter smooth muscle tone by hyperpolarizing VSMC E_m (1, 2, 41). Several mechanisms have been proposed to explain NO regulation of E_m. NO can elicit hyperpolarization both indirectly through activation of cGMP-dependent protein kinase and directly through opening of K^+ channels. Specifically, cGMP-dependent protein kinase has been shown to increase the open probability of BKCa channels (5) and K ATP channels (37), whereas others

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have demonstrated that NO directly stimulates BK\textsubscript{Ca} channel opening (2, 27). These effects of NO are thought to contribute to its vasodilator actions.

We have reported previously that inhibition of NO synthase (NOS) with \textsuperscript{N}^-\textsuperscript{nitro}-l-arginine (\textsuperscript{l}-NNA) for 2 wk results in elevated vascular smooth muscle sensitivity to depolarization-induced contraction (16). Because NO can directly activate K\textsuperscript{+} channels and hyperpolarize arterial smooth muscle cells, and because hypertension has been associated with inhibition of certain K\textsuperscript{+} channels, we hypothesized that inhibition of NO production in \textsuperscript{l}-NNA hypertensive rats (LHR) decreases K\textsuperscript{+} channel activation in VSMCs. In the presence of other depolarizing conductances, this would lead to a consequent depolarization of \(E_m\) and increased sensitivity to contractile agents that depend on VDCC activation. This hypothesis was tested in VSMCs from LHR and control rats.

**METHODS**

**Animals.** Male Sprague-Dawley rats (200–300 g) were divided into two groups: LHR and control. The LHR group drank water containing \textsuperscript{l}-NNA (0.5 g/l), whereas control rats drank water alone. Systolic blood pressure was measured using an indirect tail-cuff method (plethysmographic detection, IITC; Woodland Hills, CA). After 2 wk of \textsuperscript{l}-NNA treatment, when systolic blood pressure was elevated in the LHR group (control = 145.3 ± 1.2 mmHg and LHR = 211.9 ± 2.6 mmHg), animals were anesthetized using pentobarbital sodium (50 mg/kg) and exsanguinated. The superior mesenteric artery was rapidly removed. Arteries isolated in PSS containing (in mM) NaCl 134, KCl 4.7, KH\textsubscript{2}PO\textsubscript{4} 1.17, MgSO\textsubscript{4} 5.5, dextrose 0.026, CaCl\textsubscript{2} 1.6, and CaNa\textsubscript{2}EDTA 1.0 at pH 7.4. Arteries were cleaned of connective tissue and surrounding fat. Depending on the protocol, artery segments were left intact or cut open longitudinally, pinned, and superfused with oxygenated PSS (Warner Instruments), and superfused with either pressurized or open, nonpressurized artery segments containing PSS bubbled with 95% O\textsubscript{2}-5% CO\textsubscript{2}. For most recordings, segments were cut open longitudinally, pinned, and superfused with 10\textsuperscript{-5} M L-NNA in ethanol, the BK\textsubscript{Ca} channel antagonist iberiotoxin (50 nM), and to increasing extracellular concentrations of [K\textsuperscript{+}]. Vehicle alone did not affect \(E_m\) in any preparation. All reagents were purchased from Sigma (St. Louis, MO).

**Data analysis and statistics.** The VSMC \(E_m\) values are reported as means ± SE. For each experimental protocol, each \(n\) is the average \(E_m\) of at least three successful individual impalements in a single tissue (stable for at least 1 min), and \(n\) represents the number of animals. Data were analyzed using two-way ANOVA, followed by the Tukey’s post hoc test for all pairwise comparisons. Significant difference was determined at the \(P < 0.05\) level.

**RESULTS**

**Baseline \(E_m\).** VSMCs in open segments of superior mesenteric arteries were significantly depolarized in arteries from LHR compared with controls (Fig. 1). Control VSMC \(E_m\) was \(-44.5 ± 1.0\) mV versus LHR \(E_m\) of \(-36.8 ± 1.0\) mV. Thus VSMCs in arteries from LHR are in a relatively depolarized state compared with control VSMCs.

**Effects of \textsuperscript{l}-NNA on \(E_m\).** Effects of acute \textsuperscript{l}-NNA administration on \(E_m\) were analyzed by adding \textsuperscript{l}-NNA (100 \textmu M) to the superfusion solution. \textsuperscript{l}-NNA caused a significant depolarization in control VSMCs with \(E_m\) changing from \(-44.5 ± 1.0\) to \(-39.0 ± 1.9\) mV. Addition of the same concentration of \textsuperscript{l}-NNA to LHR VSMCs did not change VSMC \(E_m\) (from \(-36.8 ± 1.1\) to \(-36.9 ± 2.0\) mV; Fig. 2A). After \textsuperscript{l}-NNA addition, \(E_m\) was not different between LHR and control VSMCs. These data suggest that a portion of the observed depolarization in VSMCs from LHR is due to persistent NOS blockade.

**Effects of endothelium removal on \(E_m\).** The contribution of endothelial cell-derived factors to \(E_m\) was assessed by comparing \(E_m\) in two segments of the same artery: one with the endothelium intact and one denuded of endothelium. Removal of the endothelium did not change \(E_m\) in VSMCs from LHR (from \(-36.9 ± 1.5\) to \(-36.0 ± 2.6\) mV); however, endothelium removal resulted in depolarization in control VSMCs (from \(-45.3 ± 1.4\) to \(-40.8 ± 2.4\) mV; Fig. 2B). Interestingly, conclusions with \textsuperscript{l}-NNA and responses to exogenous addition of SNAP 27.

VSMCs were impaled with 3 M KCl-filled microelectrodes with tip resistances of 60–100 M\textohm, and \(E_m\) was measured using standard intracellular techniques. Briefly, \(E_m\) was measured using a high-input impedance amplifier (WPI Electro 705), filtered at 100 Hz, and recorded either alone or simultaneously with intra-arterial pressure (Gould P-3 pressure transducer) on a dual-channel chart recorder (Gould Scientific; Cleveland, OH) with Axoscope (Axon Instruments; Union City, CA) software. Intracellular recordings were accepted only if 1) \(E_m\) measurements exhibited sharp negative deflections upon cell penetration; 2) a stable recording was held for at least 1 min before experimental manipulations; and 3) an abrupt return to baseline was observed upon withdrawal of the electrode. \(E_m\) was measured continuously both before and during the addition of vehicle or drug.

**Chemicals.** \(E_m\) was recorded in response to superfusion with the NO donor SNAP (10 \textmu M in ethanol), \textsuperscript{l}-NNA (10 \textmu M in PSS), \textsuperscript{l}-arginine (30 mM in PSS), the BK\textsubscript{Ca} channel agonist NS-1619 (100 \textmu M in ethanol), the BK\textsubscript{Ca} channel antagonist iberiotoxin (50 nM), and to increasing extracellular concentrations of [K\textsuperscript{+}]. Vehicle alone did not affect \(E_m\) in any preparation. All reagents were purchased from Sigma (St. Louis, MO).
this response was similar to that produced by chronic NOS inhibition. In addition, after endothelium removal, $E_m$ was not different between groups. These data suggest that removal of the endothelial layer has a similar effect to treatment with L-NNA, presumably by removing an endogenous hyperpolarizing factor such as NO.

**Effects of L-NNA and L-arginine on $E_m$.** The ability to reverse the NOS inhibition-induced depolarization was assessed in two separate experiments by adding excess NOS substrate L-arginine to the bathing solution. The first experiment assessed the ability of L-arginine to reverse depolarization after acute exposure to L-NNA. After $E_m$ was measured, segments were treated with 100 μM L-NNA and then exposed to L-arginine to determine whether acute L-NNA-induced depolarization could be reversed. In three of four arteries, L-arginine (30 mM) successfully reversed the L-NNA-induced depolarization (data not shown).

A second set of related experiments evaluated the ability of L-arginine to reverse chronic NOS inhibition depolarization of VSMCs in LHR arteries. Arteries were incubated with L-arginine (30 mM) for 30 min before $E_m$ was recorded. L-Arginine hyperpolarized VSMCs from both control rats and LHR. $E_m$ of VSMCs from LHR exposed to L-arginine was not different from $E_m$ of VSMCs from control rats in PSS (control: $-44.5 \pm 1.0$ mV vs. L-NNA plus L-arginine: $-45.8 \pm 2.1$ mV). However, after L-arginine, VSMCs from LHR were still depolarized compared with control VSMCs treated with L-arginine ($-45.8 \pm 2.1$ vs. $-55.4 \pm 2.5$ mV, respectively; Fig. 3A). The remaining $E_m$ difference between the groups after treatment with L-arginine suggests that decreased NOS activity does not fully account for the difference observed in resting $E_m$ and indicates that a non-NOS component may contribute to the depolarization in LHR arteries.

**Effects of SNAP on $E_m$.** The ability of a NO donor (10 μM SNAP) to hyperpolarize VSMCs was evaluated by adding SNAP or its vehicle to the bath after cell impalement. Addition of SNAP hyperpolarized VSMCs in open segments from LHR but did not significantly change the $E_m$ of VSMCs from control animals. In the presence of SNAP, there was no significant difference in the $E_m$ between LHR ($-44.5 \pm 2.1$ mV) and control cells ($-48.0 \pm 2.5$ mV; Fig. 3B). Thus the NO donor caused a greater hyperpolarization in LHR VSMCs, suggesting that endogenous NO activation of $g_K$ is greater in control VSMCs. Therefore, VSMCs from LHR animals, which exhibited a larger NO-dependent...
LHR VSMCs more than control VSMCs. In the presence of exogenous acetyl penicillamine (SNAP) or its vehicle, SNAP hyperpolarized

...that the BKCa channel agonist NS-1619 hyperpolarized LHR and control rats. 

channels increased the bathing solution to determine whether L-arginine restores LHR

to control values. Em was recorded in segments incubated in physiological saline solution with L-arginine or vehicle for 30 min before the recording began. B: Em in segments from control rats (n = 9) and LHR (n = 9) after the addition of the NO donor S-nitroso-N-acetyl penicillamine (SNAP) or its vehicle. SNAP hyperpolarized LHR VSMCs more than control VSMCs. In the presence of exogenous NO, Em between groups was not different. Vehicle did not affect Em in any cells. *Significantly different from vehicle; #significantly different from controls within group.

hyperpolarization, may possess fewer open NO-sensitive K⁺ channels.

BKCa channel agonist NS-1619 hyperpolarization. The BKCa channel agonist NS-1619 (7, 33) was used to determine whether maximal activation of these channels increased Em more in VSMCs from LHR, similar to the response to exogenous NO. NS-1619 or its vehicle was added to the bath after cell impalement, and responses to NS-1619 were measured at their maximum. Addition of NS-1619 caused hyperpolarization in VSMCs from both the control and LHR groups (Fig. 4A). NS-1619 hyperpolarized cells in both groups by a similar amount (control: from −50.8 ± 1.0 to −62.5 ± 1.2 mV and LHR: from −43.7 ± 1.1 to −55.6 ± 1.2 mV), so that the Em in the presence of the agonist was still different between LHR and control VSMCs. The Em difference in the presence of the BKCa agonist suggests that decreased activation of BKCa channels does not explain the difference in Em between VSMCs from LHR and control rats.

Iberiotoxin-induced depolarizations. The observation that the BKCa channel agonist NS-1619 hyperpolarized both control and LHR VSMCs by a similar magnitude suggests that activity and/or expression of these channels are not changed during l-NNA-induced hyperten-
fixed to keep pH constant. $E_m$ versus $[K^+]_o$ were fit using a least-squares linear regression for $[K^+]_o \approx 3.0$ mM.

Increases in $[K^+]_o$ decrease the electrochemical potential for $K^+$ and move potassium equilibrium potential ($E_K$) to a more positive value. Increased $[K^+]_o$ depolarized VSMCs approximately linearly for $[K^+]_o$ above 3.0 mM (Fig. 5). VSMCs from control rats had a steeper slope of the $E_m$ versus $[K^+]_o$, than did those from LHR, suggesting that $g_K$ makes a larger contribution to $E_m$ in VSMCs from control rats (slope = $27.7 \pm 1.3 \text{ mV/log} [K]_o$ mM for control and $20.2 \pm 1.7 \text{ mV/log} [K]_o$ mM for LHR, $P < 0.05$).

**Pressurized data.** To validate the physiological relevance of NO regulation of $E_m$, mesenteric segments were pressurized and $E_m$ was recorded under basal conditions and after the addition of l-NNA and SNAP. Pressurizing the artery segments depolarized VSMCs in arteries from both groups compared with pinned segments (Fig. 6A). $E_m$ for VSMCs from control animals was $-41.8 \pm 1.0$ mV in pressurized segments and $-32.6 \pm 1.4$ mV in pressurized arteries from LHR. Thus VSMCs are still depolarized in LHR arteries after pressurization, and the mean difference in $E_m$ between groups becomes more pronounced with luminal pressure.

Effects of acute l-NNA administration on $E_m$ were analyzed by adding l-NNA (100 $\mu$M) to the internal perfusion solution pressurizing the vessel for 30 min before recording. l-NNA in pressurized control vessels produced a depolarization similar to that observed in open segments (from $-41.2 \pm 1.1$ to $-34.3 \pm 1.3$ mV). Also similar to open segments, the addition of the same concentration of l-NNA to pressurized segments from LHR arteries did not cause depolarization (from $-32.7 \pm 0.9$ to $-32.9 \pm 1.3$ mV; Fig. 6B). In the presence of l-NNA, $E_m$ of pressurized vessels was not different between groups. These data strongly suggest that a portion of the observed depolarization in VSMCs from LHR is due to persistent NOS blockade.

Addition of SNAP to pressurized vessels caused a significant hyperpolarization in both control and LHR vessels (control: from $-42.8 \pm 0.5$ to $-48.1 \pm 0.6$ mV and LHR: from $-33.76 \pm 0.5$ to $-46.0 \pm 0.5$ mV).
However, in pressurized vessels treated with SNAP, there was still a significant difference in $E_m$ between LHR ($-46.0 \pm 0.5$ mV) and control cells ($-48.1 \pm 0.6$ mV; Fig. 6C). Therefore, similar to data from open segments, the larger NO-dependent hyperpolarization of LHR pressurized arteries further supports the conclusion that LHR VSMCs may possess fewer open NO-sensitive $K^+$ channels.

**DISCUSSION**

The major findings of the present study are as follows. First, VSMCs of mesenteric arteries from LHR were depolarized close to the potential for activation of VDCCs, $-30$ to $-40$ mV (9, 36). Second, NO removal, by endothelial cell removal or addition of l-NNa, only depolarized VSMCs from control rats. Third, increased NO availability, either endogenously (by adding l-arginine) or exogenously (by adding SNAP) hyperpolarized cells from both groups. Fourth, the NO donor SNAP hyperpolarized LHR VSMCs more than controls. Fifth, the BKCa channel agonist NS-1619 hyperpolarized VSMCs from both control rats and LHR, whereas the BKCa channel antagonist iberiotoxin depolarized VSMCs from both LHR and control rats. However, in the presence of the antagonist or agonist, $E_m$ was still different between the two groups. Sixth, increasing $[K^+]_o$, depolarized $E_m$ in an approximately Nernstian manner with a steeper slope for control VSMCs, suggesting that $g_K$ makes a larger contribution to $E_m$ in VSMCs from control rats. Finally, pressurizing superior mesenteric arteries caused a depolarization of $7\sim10$ mV and augmented the difference in $E_m$ between control and LHR VSMCs. In addition, responses to l-NNa or SNAP were qualitatively similar in open and pressurized segments. These important and novel findings are, to our knowledge, the first report of altered $E_m$ regulation in NO inhibition hypertension and the first to demonstrate that in vivo NOS inhibition elicits $E_m$ depolarization.

The purpose of this study was to determine whether membrane depolarization, resulting from decreased NO-stimulated $g_K$, could account for the increased contractile sensitivity to $\alpha_2$-adrenergic agonists and to the depolarization-induced contraction reported previously for mesenteric arteries from NOS inhibition-treated rats (16). Many studies have reported a lack of contractile responses to $\alpha_2$-agonists except in the presence of depolarizing agents. Therefore, depolarization of $E_m$ could account for the increased $\alpha_2$-adrenergic agonist sensitivity in NOS inhibition hypertension. On the basis of these observations, we hypothesized that increased contractile sensitivity of vascular smooth muscle to $\alpha_2$-adrenergic agonists in arteries from NOS-inhibited rats is caused in part by VSMC depolarization. Our measurements of $E_m$ in both pressurized and open arteries support this hypothesis and show that VSMCs from LHR display an $E_m$ that is $\sim9$ mV more depolarized than that of VSMCs from control rats. In agreement with our findings, several other studies have demonstrated VSMC depolarization in the mesenteric bed in other models of hypertension (3, 8, 10). We report here that $E_m$ in mesenteric artery VSMCs from LHR are depolarized to a level near the activation potential for VDCCs (9, 36). This was true in both open and pressurized artery segments, suggesting that membrane depolarization contributes to elevated arterial contractile responses during systemic NOS inhibition.

VSMC depolarization has been observed and attributed to changes in membrane conductances including decreased $g_K$ (24), increased calcium conductance (20), or increased chloride conductance (12) in several models of hypertension. Previous studies demonstrating that VSMCs are depolarized in arteries from hypertensive animals suggest that elevated arterial pressure may directly modulate ion channel function or expression to depolarize VSMCs. In addition, it has been shown that endothelial derived factors, including NO, endothelium-derived hyperpolarizing factor, and prostaglandins, hyperpolarize smooth muscle through activation of $g_K$. Therefore, loss of hyperpolarizing endothelial factors in hypertension could also be responsible for the depolarization. In the present study, endothelial cell removal and exposure to exogenous l-NNa were used to separate the contribution of loss of endothelial NO activation of $K^+$ channels and VSMC loss of channel expression to the observed depolarization in LHR VSMCs. That is, if the change in $E_m$ is caused by loss of NO, then eliminating NO synthesis should cause control VSMCs to have an $E_m$ that is similar to LHR VSMC $E_m$. If the change was intrinsic to VSMCs, then there should still be a difference in $E_m$, even in the absence of NO synthesis in control arteries.

Acute exposure to l-NNa or removal of the endothelium depolarized only control VSMCs, suggesting that the depolarization of $E_m$ observed in LHR VSMCs is caused mostly by the loss of endothelial NO production. Indeed, addition of a NOS inhibitor directly to control vascular segments depolarized VSMCs, as has been reported by other investigators (29, 35). Similarly, depolarization of VSMCs after endothelium removal has also been observed previously (11). In this study, endothelium removal caused depolarization only in control arteries, indicating that basal release of endothelial hyperpolarizing factors contributes to VSMC $E_m$ in these vessels. This depolarization is presumably due to the loss of NO activation of $g_K$. However, $E_m$ was not altered by removing the endothelium from LHR arteries and was still depolarized compared with control VSMCs even in the absence of endothelium. This suggests that there is an intrinsic change in VSMCs that contributes to the depolarization of $E_m$ in VSMCs from LHR.

l-Arginine reversal of l-NNa-induced depolarization further supports the hypothesis that endothelial cell production of NO contributes to resting $E_m$. This was observed for blockade of NO with either acute or chronic exposure to l-NNa and suggests that loss of NO activation of $g_K$ can explain in part the depolarization in LHR VSMCs. l-Arginine restored $E_m$ in LHR
VSMCs close to the resting $E_m$ of VSMCs from control rats. However, even after exposure to 300 times more L-arginine than L-NNA (30), VSMCs from LHR were still depolarized compared with control VSMCs exposed to the same L-arginine concentration. This persistent difference in $E_m$ between the two groups suggests that a non-NOS-dependent component contributes to the observed depolarization.

One potential mechanism for NO-mediated hyperpolarization is activation of K\textsuperscript{+} channels. Direct application of exogenous NO to isolated arteries has been shown to cause hyperpolarization and vasodilation through activation of BK\textsubscript{Ca} channels (2, 19) as well as other K\textsuperscript{+} channels (28, 29). By this mechanism, chronic inhibition of NOS could cause VSMC depolarization by removing an endogenous activator of BK\textsubscript{Ca} channels. Under these conditions, replacing NO should restore $E_m$ to control levels. In support of this, the NO donor SNAP caused a significant hyperpolarization only in VSMCs from LHR, suggesting that in the control tissue, NO activation of $g_K$ is at its maximum. Therefore, VSMCs from LHR appear to have fewer NO-sensitive K\textsuperscript{+} channels open.

BK\textsubscript{Ca} channels contribute significantly to resting $E_m$ in VSMCs and provide a mechanism to oppose depolarization and subsequent vasoconstriction (4, 31). Addition of the BK\textsubscript{Ca} channel agonist NS-1619 (7, 33) was shown to cause hyperpolarization and vasodilation in VSMCs and provide a mechanism to oppose depolarization in LHR VSMCs, suggesting that the altered conductance(s) responsible for depolarization in the LHR arteries are not affected by stretch.

In summary, the current study demonstrates that VSMCs are depolarized in mesenteric arteries from LHR. This may contribute to augmented contractile sensitivity and thus to the elevated peripheral resistance in NOS inhibition hypertension. This depolarization is due in part to loss of NO activation of $g_K$ but does not appear to result entirely from a loss of BK\textsubscript{Ca} conductances. These results suggest that endothelial dysfunction resulting in decreased NO production may be the underlying factor in at least some of the vascular hypersensitivity in NO-deficient hypertension.

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