Nitric oxide synthase inhibition activates L- and T-type Ca\(^{2+}\) channels in afferent and efferent arterioles

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NITRIC OXIDE (NO) is synthesized from the amino acid l-arginine by a constitutive endothelial nitric oxide synthase (NOS) in endothelial cells and by neuronal NOS in macula densa cells. NO from both sources contributes to the regulation of the renal vasculature (20, 31, 32). It modulates constrictor responses of afferent and efferent arterioles (8, 19, 23) and contributes to the regulation of renal medullary blood flow (29) via activation of guanylyl cyclase and other signaling mechanisms (38). NO can influence voltage-activated Ca\(^{2+}\) channels either directly or indirectly (3, 6). Inhibition of NOS causes afferent arteriolar vasocstriction, which has been associated with an increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)])\(_i\) in vascular smooth muscle cells (VSM) (45), but the mechanisms responsible for the increased [Ca\(^{2+}\)])\(_i\) have not been clearly determined.

T-type Ca\(^{2+}\) channels are molecularly and functionally expressed in both pre- and postglomerular arterioles, but L-type Ca\(^{2+}\) channels exert their effects predominantly on preglomerular arterioles (4, 10, 34, 43). High-voltage-activated (HVA) L-type and low-voltage-activated (LVA) T-type Ca\(^{2+}\) channel currents have been observed in VSM cells isolated from interlobar and arcuate arteries of rat kidney (13) and from afferent arterioles of mouse kidney (39). T-type Ca\(^{2+}\) channels CaV 3.1 and CaV 3.2 are expressed in the juxtaglomerular afferent and efferent arterioles of rat kidney (15). L-type Ca\(^{2+}\) channel blockers also block afferent arteriolar vasoconstriction responses to ANG II (4, 43). In contrast, the ability of ANG II to constrict efferent arterioles is not diminished by addition of L-type Ca\(^{2+}\) channel blockers (4, 11, 43). In micropuncture studies, L-type Ca\(^{2+}\) channel blockade also decreased efferent arteriolar vascular resistance only in spontaneously hypertensive rats (SHR) chronically treated with N\(^{\text{G}}\)-nitro-l-arginine methyl ester (l-NAME), a NOS inhibitor, but not in untreated SHR or in normotensive rats (22, 30). In addition, NOS inhibition with N-nitro-l-arginine (l-NNA) decreased resting efferent and afferent arteriolar diameters in normotensive (20, 32) and ANG II-infused hypertensive rat kidneys (19). Administration of minostradiol, a T-type Ca\(^{2+}\) channel blocker, was shown to cause dilation of efferent arterioles in vivo in dogs (17), block the ANG II-induced efferent arteriolar constriction in the isolated, perfused hydronephrotic rat kidney (33), and reduce the afferent and efferent arteriolar resistances which had been increased by chronic administration of l-NAME in drinking water for 3 wk to SHR (30). It was also demonstrated that pimozide and mibefradil, potent T-type Ca\(^{2+}\) channel blockers (1, 2, 7, 10, 34, 36), vasodilate both afferent and efferent arterioles in the isolated blood-perfused juxtaglomerular nephron preparation of Sprague-Dawley rats (10), indicating that T-type Ca\(^{2+}\) channels are functionally expressed in both afferent and efferent arterioles of the kidney.

NOS inhibition (1, 10, and 100 \(\mu\)mol/l) of LCC activity, 55 mmol/l KCl, which depolarizes and constricts AA, efferent (0.4 \(\mu\)mol/l) of diltiazem to block EA constriction caused by L-NNA contrasts with the lack of efferent effects in resting and SNAP-treated l-NNA-preconstricted arterioles and during ANG II-mediated vasoconstriction, suggesting a recruitment of LCC in EA when NOS is inhibited. These data help explain how endothelial dysfunction associated with hypertension may lead to enhanced activity of LCC in postglomerular arterioles and increased postglomerular resistance.
afferent and efferent arterioles. However, the roles of T- and L-type Ca\(^{2+}\) channels in the vasoconstriction elicited by inhibition of NOS have not been established (20, 30, 32).

The present study was performed to determine the relative contributions of T- and L-type Ca\(^{2+}\) channels in mediating Ca\(^{2+}\) entry responsible for afferent and efferent arteriolar vasoconstriction following NOS inhibition. We used the in vitro blood-perfused juxtamedullary nephron technique combined with videomicroscopy and tested the responses to NOS inhibition in the presence of a selective L-type Ca\(^{2+}\) channel blocker, diltiazem (12, 40), and a potent T-type Ca\(^{2+}\) channel blocker, pimozide. We chose pimozide as a T-type Ca\(^{2+}\) channel blocker for the present study because previous studies, including ours, demonstrated that pimozide is a more potent relatively selective T-type Ca\(^{2+}\) channel blocker (1, 2, 7, 10, 34, 36). It has been shown that pimozide is the most potent T-type channel blocker among several neuroleptics (36); mibefradil was less potent than pimozide at blocking various T-type channels; however, pimozide failed to prevent the strong depolarization induced by high KCl indicating that pimozide does not exert substantive blockade on L-type channels (10).

MATERIALS AND METHODS

The experimental protocols and procedures were approved by the Tulane University Institutional Animal Care and Use Committee. As previously described (4, 10, 11), afferent and efferent arteriolar diameters were measured in vitro using the isolated blood-perfused juxtamedullary nephron technique combined with videomicroscopy.

Experiments were made in Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 370–410 g. Rats were anesthetized with pentobarbital sodium (50 mg/kg ip), and a cannula was inserted in the left carotid artery for blood collection. Blood was collected in a heparinized (500 U) syringe via the carotid arterial cannula and centrifuged to separate the plasma and cellular fractions. Theuffy coat was removed and discarded. After sequential passage of the plasma through 5.0- and 0.22-μm filters (Gelman Sciences, Ann Arbor, MI), erythrocytes were added to achieve a hematocrit of 35%. This reconstituted blood was passed through a 5-μm nylon mesh and thereafter stirred continuously in a closed reservoir that was pressurized with a 95% O\(_2\)-5% CO\(_2\) gas mixture. The right kidney was perfused through a cannula inserted in the superior mesenteric artery and advanced into the right renal artery. The perfusate was a Tyrode’s solution (pH 7.4) containing 5.1% BSA and a mixture of L-amino acids thereafter stirred continuously in a closed reservoir that was pressurized with a 95% O\(_2\)-5% CO\(_2\) gas mixture. The kidney was excised and sectioned longitudinally, and the papilla was retained intact with the perfused dorsal two-thirds of the organ. The papilla was reflected to expose the pelvic mucosa and tissue covering the inner cortical surface. Overlying tissue was removed to expose the tubules, glomeruli, and related vasculature of the juxtamedullary nephrons. The arterial supply of the exposed microvasculature was isolated by ligating the larger branches of the renal artery. After the dissection was completed, the Tyrode’s perfusate was replaced with the reconstituted blood. Perfusion pressure was monitored by a pressure catheter centered in the tip of the perfusion cannula. Renal perfusion pressure was regulated by adjusting the rate of gas inflow into the blood reservoir and set at 100 mmHg. The inner cortical surface of the kidney was continuously superfused with a warmed (37°C) Tyrode’s solution containing 1% BSA. The tissue was transilluminated on the fixed stage of a microscope (Nikon) equipped with a water-immersion objective (X40). Video images of the microvessels were transferred by a Newvicon camera (model NC-67M; Dage-MTI, Michigan City, IN) through an image enhancer (model MFI-1452; MFI Enterprises, Starkville, MS) to a video monitor (Conrac Display Systems, Covina, CA). The video signal was recorded on videotape for later analysis. Afferent and efferent arteriolar inside diameters were measured at 30-s intervals using a calibrated digital imagesharing monitor (Instrumentation for Physiology and Medicine, San Diego, CA). Single afferent or efferent arterioles were visualized. Treatments were administered by superfusing the tissue with a Tyrode’s solution containing the agent to be tested or vehicle. Experimental protocols. Afferent and efferent arteriolar diameters were measured within 100 μm of the glomerulus. Efferent arterioles were studied between the glomerulus and the first bifurcation. The efferent arterioles that we studied are from juxtamedullary nephrons and generally give rise to vasa recta. For each experiment, a single afferent or efferent arteriole that showed rapid blood flow was selected for study. After a 10-min equilibration period, an experimental protocol was initiated consisting of consecutive 10- to 15-min treatment periods. Steady-state diameter determinations were calculated from the average of measurements obtained during the final 5 min of each 10-min treatment period. Initial experiments were performed to determine the effects of the T-type Ca\(^{2+}\) channel blocker, pimozide, on L-NNA-induced constriction of afferent and efferent arterioles. Afferent and efferent arteriolar inside diameters were measured during sequential exposure of the kidney to vehicle, then to 0.1, 10, and 100 μmol/l L-NNA alone or in the presence of pimozide (10 μmol/l). A second series of experiments was performed to determine the effects of L-type Ca\(^{2+}\) channel blockade with diltiazem, on L-NNA-induced constriction of afferent and efferent arterioles and the synergy or overlap between the effects of T-type and L-type Ca\(^{2+}\) channel blockade.

Arteriolar inside diameter was measured during sequential exposure of the vessel to superfuse solutions of various compositions as follows: 1) control vehicle, 2) 100 μmol/l L-NNA, 3) 100 μmol/l L-NNA plus 10 μmol/l diltiazem, and 4) 100 μmol/l L-NNA plus 10 μmol/l diltiazem and 10 μmol/l pimozide. Previous data indicate that the lowest pimozide and diltiazem concentrations that provide the most effective dilation is 10 μmol/l (4, 10, 11). In addition, the effects of diltiazem on L-NNA-preconstricted and resting efferent arterioles were compared. The third experimental protocol was performed to determine the effect of diltiazem under condition where the L-NNA-induced effenter constriction was reversed by superfusion with a NO donor, S-nitroso-N-acetylpenicillamine (SNAP). L-NNA (100 μmol/l) preconstricted efferent arterioles were treated with SNAP (10 μmol/l), and the effects of diltiazem (10 μmol/l) were tested to determine whether enhanced NO levels reduced L-type Ca\(^{2+}\) channel activity in efferent arterioles. The fourth experimental protocol was performed to determine the response in resting and L-NNA (100 μmol/l) preconstricted efferent arterioles to high KCl and norepinephrine (NE). KCl activates HVA L-type Ca\(^{2+}\) channels; however, NA stimulates α-adrenergic receptors, causing the release of Ca\(^{2+}\) from intracellular stores (9, 21, 25). Experiments involved a control period followed by a 10-min exposure to an isotonic solution containing 55 mM KCl then a 5-min exposure to 55 mM KCl plus 1 μmol/l NE. The resting or L-NNA-preconstricted efferent arteriolar responses to KCl and NE were assessed. The superfusion solution was modified by replacing part of the NaCl with KCl but maintaining the original osmolality and all the other constituents in the normal Tyrode’s solution. Pimozide, diltiazem, L-NNA, and NE were obtained from Sigma (St. Louis, MO).

Statistical analysis. All data are reported as means ± SE. Data were analyzed by two-way ANOVA or one-way ANOVA, followed by a Bonferroni’s multiple-comparison post hoc test. Values of P < 0.05 were considered statistically significant.
RESULTS

Effects of pimozide on L-NNA-induced constriction of afferent and efferent arterioles. Figure 1 illustrates the effects of pimozide on the afferent and efferent arteriolar diameter responses to L-NNA. In response to the three concentrations of L-NNA (1, 10, and 100 μmol/l), afferent arteriolar diameter decreased significantly by 6.0 ± 0.3, 13.7 ± 1.7, and 19.9 ± 1.4%, respectively (n = 5, P < 0.01). Superfusion with solutions containing pimozide (10 μmol/l) caused significant dilation of afferent arterioles, with average diameter increasing from 17.8 ± 1.3 to 22.4 ± 0.6 μm (n = 6, P < 0.01) for an increase in afferent arteriole diameter of 26.0 ± 2.7%. In the presence of pimozide (10 μmol/l), afferent arteriolar diameter responses to L-NNA were markedly attenuated and the decreases in diameter were not significant (0.9 ± 0.6, 1.5 ± 0.5, and 1.7 ± 0.5%, P < 0.01 vs. L-NNA alone group, n = 6). Efferent arteriolar diameter also decreased significantly by 6.2 ± 0.5, 13.3 ± 1.1, and 19.0 ± 1.9% (n = 5, P < 0.01) during superfusion of 1, 10, and 100 μmol/l L-NNA, respectively. In the presence of pimozide (10 μmol/l), efferent arteriolar diameter increased significantly and the responses to L-NNA were markedly attenuated averaging 0.4 ± 0.1, 2.1 ± 0.7, and 2.5 ± 1.0% (P < 0.01 vs. L-NNA alone group, n = 5).

In both cases, the responses to L-NNA alone were significantly greater than the responses to L-NNA during concomitant treatment with pimozide. Furthermore, the L-NNA-induced effects in the presence of pimozide were not statistically significant. Thus L-NNA-induced constriction of afferent and efferent arterioles was markedly inhibited by pimozide.

Effects of diltiazem on L-NNA-induced constriction of afferent and efferent arterioles and the synergy or overlap between the effects of L-type (LCC) and T-type Ca²⁺ channels (TCC) blockers. L-NNA (100 μmol/l) caused significant constriction of afferent (n = 5) and efferent (n = 6) arterioles, which was significantly inhibited by adding diltiazem (10 μmol/l). Pimozide (10 μmol/l) superimposed on diltiazem did not elicit further dilation of afferent arterioles (n = 5) and only caused a slight and nonsignificant increase of efferent arteriolar diameters (n = 5). Values are means ± SE. **P < 0.01 compared with L-NNA alone. ###P < 0.01 compared with control condition.

Fig. 1. Effects of pimozide on N-nitro-L-arginine (L-NNA)-induced constriction of afferent and efferent arterioles. Pimozide (10 μmol/l) markedly inhibited the L-NNA-induced contraction of afferent (n = 6) and efferent arterioles (n = 5). Diameters expressed as μm were measured with renal arterial pressures maintained at 100 mmHg. Values are means ± SE. *P < 0.05, **P < 0.01 compared with baseline. ###P < 0.01 compared with pimozide-treated group.

Fig. 2. Effects of diltiazem on L-NNA-induced constriction of afferent and efferent arterioles and the synergy or overlap between the effects of L- and T-type Ca²⁺ channel blockers. The afferent and efferent arteriolar responses to L-NNA, diltiazem, and pimozide are shown in Fig. 2. L-NNA (100 μmol/l) caused constriction of afferent and efferent arterioles with average diameters decreasing from 18.6 ± 0.3 to 14.9 ± 0.4 μm, for a decrease of 19.6 ± 3.5% (n = 5, P < 0.01) and from 19.2 ± 0.4 to 16.1 ± 0.6 μm, for a decrease of 15.1 ± 2.1% (n = 6, P < 0.01), respectively. L-NNA-induced constriction was significantly inhibited by adding diltiazem 10 μmol/l. In the L-NNA-constricted arterioles, diltiazem increased afferent and efferent arteriolar responses to L-NNA. In the presence of pimozide (10 μmol/l), afferent arteriolar diameter responses to L-NNA were markedly attenuated and the decreases in diameter were not significant (0.9 ± 0.6, 1.5 ± 0.5, and 1.7 ± 0.5%, P < 0.01 vs. L-NNA alone group, n = 6). Efferent arteriolar diameter also decreased significantly by 6.2 ± 0.5, 13.3 ± 1.1, and 19.0 ± 1.9% (n = 5, P < 0.01) during superfusion of 1, 10, and 100 μmol/l L-NNA, respectively. In the presence of pimozide (10 μmol/l), efferent arteriolar diameter increased significantly and the responses to L-NNA were markedly attenuated averaging 0.4 ± 0.1, 2.1 ± 0.7, and 2.5 ± 1.0% (P < 0.01 vs. L-NNA alone group, n = 5).

In both cases, the responses to L-NNA alone were significantly greater than the responses to L-NNA during concomitant treatment with pimozide. Furthermore, the L-NNA-induced effects in the presence of pimozide were not statistically significant. Thus L-NNA-induced constriction of afferent and efferent arterioles was markedly inhibited by pimozide.
eff erent arteriolar diameters by 36.3 ± 6.4% \((n = 5, P < 0.01)\) and 17.1 ± 1.8% \((n = 6, P < 0.01)\), respectively. The concomitant treatment with pimozide \((10 \mu \text{mol/l})\) and diltiazem \((10 \mu \text{mol/l})\) did not elicit further dilation than that caused by diltiazem alone with afferent arteriolar diameters changing from 20.2 ± 0.7 to 20.3 ± 0.9 μm \((n = 5, P > 0.05)\) and only caused a slight further increase in efferent arteriolar diameters that was not statistically significant, changing 3.0 ± 0.9% from 18.9 ± 0.6 to 19.6 ± 0.5 μm \((n = 5, P > 0.05)\). Thus L-NNA-induced constriction of afferent or efferent arterioles was markedly blocked by diltiazem, and pimozide superimposed on diltiazem did not elicit further dilation of afferent and efferent arterioles.

**Efferent arteriolar response to L-NNA followed by treatment with SNAP and diltiazem.** Figure 3 illustrates the response to diltiazem in L-NNA-preconstricted efferent arterioles during treatment with SNAP to restore NO levels. L-NNA \((100 \mu \text{mol/l})\) constricted efferent arterioles, and the vasoconstriction was reversed by adding SNAP \((10 \mu \text{mol/l})\), with average diameter increasing from 16.3 ± 0.6 to 19.7 ± 0.7 μm \((n = 5, P < 0.01)\) for an increase in efferent arteriolar diameter of 21.5 ± 2.3%. Adding diltiazem \((10 \mu \text{mol/l})\) to the SNAP-containing solutions only caused a slight and nonsignificant increase in efferent arteriolar diameter, changing from 19.7 ± 0.7 to 20.5 ± 0.8 μm \((P > 0.05)\). Thus, after NO was restored by SNAP, diltiazem exerted only modest or nonsignificant effects on L-NNA-preconstricted efferent arterioles.

**Comparison of the effects of diltiazem and pimozide on resting and L-NNA-preconstricted efferent arteriolar diameters.** Figure 4 illustrates the responses to diltiazem and pimozide in resting and L-NNA-preconstricted efferent arterioles. As previously shown, diltiazem only caused a slight and nonsignificant change in resting efferent arterioles with diameters increasing by 2.8 ± 0.7% \((n = 6, P > 0.05)\). However, in efferent arterioles treated with L-NNA, diltiazem caused a much greater dilation, with average diameter increasing from 16.1 ± 0.6 to 18.9 ± 0.5 μm \((n = 6, P < 0.01)\) for an increase in arteriole diameter of 17.1 ± 1.8%. In L-NNA-preconstricted efferent arterioles during treatment with SNAP \((10 \mu \text{mol/l})\), diltiazem only caused a modest or nonsignificant dilation, with average diameter increasing by 3.8 ± 0.8% \((n = 5, P > 0.05)\). In contrast, superfusion with solutions containing pimozide \((10 \mu \text{mol/l})\) caused significant dilation of resting efferent arterioles, with average diameter increasing from 19.4 ± 0.2 to 23.3 ± 0.6 μm \((n = 6, P < 0.01)\) for an increase in efferent arteriole diameter of 20.0 ± 2.0%. Thus diltiazem dilated the L-NNA-preconstricted efferent arterioles but did not cause significant dilation of L-NNA-treated efferent arterioles after restoration of NO by adding NO donor, SNAP, and, unlike pimozide, diltiazem had no significant effect on resting efferent arterioles.

**Effects of high KCl and NE on resting and L-NNA-preconstricted efferent arteriolar diameters.** Because the results with L-NNA contrasted with those previously observed with ANG II \((4, 11)\), further studies were done to test the effects of a depolarizing concentration high-KCl concentration on efferent arteriolar diameters during control condition and following L-NNA exposure. The resting and L-NNA-preconstricted efferent arteriolar responses to high KCl and NE are shown in Fig. 5. Superfusion with solutions containing KCl \((55 \mu \text{mol/l})\) caused only slight sustained constriction of resting efferent arterioles, with average diameters decreasing from 19.6 ± 0.4 to 17.9 ± 0.5 μm, for a decrease of 8.7 ± 1.3% \((n = 5, P < 0.05)\). Interestingly, superfusion with solutions containing KCl \((55 \mu \text{mol/l})\)-containing solutions elicited further strong constriction of both resting and L-NNA-preconstricted efferent arterioles during diameters decreasing to 11.2 ± 1.1 μm, for a decrease of 42.7 ± 5.9% \((n = 5, P < 0.01)\) and to 8.6 ± 0.3 μm, for a decrease of 83.7 ± 2.3% \((n = 5, P < 0.01)\).
arterioles, but a strong constriction in L-NNA-preconstricted high KCl caused only a modest constriction in resting efferent arterioles. Thus, unlike NE, the depolarizing solution of KCl-containing solutions elicited further strong constriction. Values are means ± SE. **P < 0.01 compared with baseline. ###P < 0.01 compared with resting group.

μm, for a decrease of 46.5 ± 3.9% (n = 5, P < 0.01), respectively. Thus, unlike NE, the depolarizing solution of high KCl caused only a modest constriction in resting efferent arterioles, but a strong constriction in L-NNA-preconstricted efferent arterioles.

DISCUSSION

NO is now recognized as having major significance in the regulation of local tone of both afferent and efferent arterioles, and inhibition of NO synthase causes sustained vasoconstriction (19, 20, 32). It is generally acknowledged that sustained vasoconstriction requires elevation of [Ca\textsuperscript{2+}]i and activation of Ca\textsuperscript{2+} channels (3, 5). Voltage-gated Ca\textsuperscript{2+} channels are perhaps the most important physiological regulator of Ca\textsuperscript{2+} entry into VSM. The present study demonstrates that the vasoconstriction of afferent and efferent arterioles from normotensive rat kidneys elicited by NO synthase inhibition with L-NNA is blocked by an L-type Ca\textsuperscript{2+} channel blocker and one T-type Ca\textsuperscript{2+} channel blocker. While the blockers that we used are among the more selective and specific agents available, it is recognized that additional studies using other L- and T-type Ca\textsuperscript{2+} channel blockers are needed to provide further support to these conclusions.

The mechanisms linking NO signaling pathways and voltage-gated Ca\textsuperscript{2+} channels remain incompletely understood. NO and NO donors cause vasorelaxation via activation of soluble guanylyl cyclase, leading to increases in intracellular cGMP concentrations in VSM (31). cGMP selectively inhibits intracellular Ca\textsuperscript{2+} release stimulated by inositol 1,4,5-triphosphate (IP\textsubscript{3}) in VSM (24, 26, 38). NO also influences voltage-gated Ca\textsuperscript{2+} channel activity. In cultured VSM cells, sodium nitroprusside (SNP), a vasodilator that provides NO, reduced [Ca\textsuperscript{2+}]i in cells in which [Ca\textsuperscript{2+}]i was elevated by depolarization. SNP also decreased current through voltage-gated calcium channels but did not affect release of calcium from intracellular stores (3). Thus the signal transduction mechanism of endothelium-dependent relaxation of VSM also involves a decrease in [Ca\textsuperscript{2+}]i, by inhibition of Ca\textsuperscript{2+} entry. In VSM cells of rat tail artery, Ca\textsuperscript{2+} entry through voltage-operated calcium channels is more sensitive to NO compared with receptor-operated calcium channels or intracellular Ca\textsuperscript{2+} release (26). Furthermore, L-NNA-induced vasoconstriction was potentiated rather than inhibited by ryanodine, an agent that inhibits VSM contraction mediated by release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum, indicating that the response was mediated by Ca\textsuperscript{2+} influx rather than by release from intracellular stores (16). NO has also been reported to inhibit L-type Ca\textsuperscript{2+} current in glomus cells of the rabbit carotid body (41), in rat insulinoma RINm5F cells (14), and in human coronary myocytes (35). In addition, NO causes dilation of afferent arterioles (44) and pulmonary arteries through activation of a Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (KCa) (37) and L-type Ca\textsuperscript{2+} channel current is suppressed by a rise in intracellular cGMP levels in VSM cells from rat portal vein (28) and mesenteric artery cells (42). Collectively, blockade of NO would be expected to reduce the cGMP-mediated inhibition of IP\textsubscript{3}, allowing increased IP\textsubscript{3}-mediated release of intracellular Ca\textsuperscript{2+}. This effect, coupled with blockade of potassium channels, might reduce membrane potential sufficiently to activate T-type Ca\textsuperscript{2+} channels which, in turn, would reduce membrane potential and further activate L-type Ca\textsuperscript{2+} channels, thus increasing entry of Ca\textsuperscript{2+}, leading to afferent and efferent arteriolar vasoconstriction. It is likely that there is a cooperativity between LVA Ca\textsuperscript{2+} channels and HVA Ca\textsuperscript{2+} channels to control the influx of Ca\textsuperscript{2+} ions in afferent and efferent arteriolar VSM cells under conditions of NO inhibition (10, 18).

A novel aspect of the present findings is that L-type Ca\textsuperscript{2+} channel blockade prevented the efferent arteriolar vasoconstriction elicited by L-NNA. The effects of L-type Ca\textsuperscript{2+} channel blockade on efferent arterioles in this setting contrast sharply with the previous findings generally failing to show effects on resting efferent arterioles or on ANG II-vasoconstricted efferent arterioles (4, 10, 11, 43). These differences suggest that endogenous NO levels exert their steady-state vasodilator influence, in part, by suppressing the activity or expression of voltage-gated Ca\textsuperscript{2+} channels. This may be due to
the effect of NO on K+ channels, which maintain the cell membrane potential relatively hyperpolarized, thus reducing the basal activity of T- and L-type Ca2+ channels. Membrane depolarization by high KCl is known to stimulate Ca2+ entry through activation of high-voltage-activated L-type Ca2+ channels; however, NE activates the α-adrenergic receptor which stimulates IP3 formation and causes the release of Ca2+ from the endoplasmic reticulum (9, 21, 25). We previously demonstrated that 55 mM KCl elicits a marked constriction of afferent arterioles, with diameter decreasing by 43.1 ± 2.6%, indicating an abundance of L-type Ca2+ channels in afferent arterioles (10). In the present study, we further found that 55 mM KCl caused only a slight constriction of resting efferent arterioles indicating a weak activity of L-type Ca2+ channels in efferent arterioles. These results further support the notion that L-type Ca2+ channels are predominantly expressed on preglomerular arterioles and normally exert only modest effects on postglomerular arterioles. Importantly, we found that high KCl caused a significantly stronger constriction when the efferent arterioles were preconstricted with L-NNA than in resting efferent arterioles. To determine the ability of an NO donor to reverse the effects of L-NNA, we performed NO donor studies. We found that after restoration of NO levels by superfusing with an NO donor, SNAP, diltiazem did not cause significant dilatation of L-NNA-treated efferent arterioles and that after restoration of NO levels by superfusing with an NO donor, SNAP-treated L-NNA efferent arterioles and the difference in responses to high KCl between resting and L-NNA-constricted efferent arterioles suggests that endogenous NO normally suppresses L-type Ca2+ channel expression or activity in efferent arterioles and that NOS inhibition leads to recruitment or activation of these usually quiescent L-type Ca2+ channels in efferent arterioles.

Thus, under condition of L-NNA-induced constriction, the effects of pimozide and diltiazem on afferent and efferent arterioles were overlapping. Furthermore, high KCl caused only a modest vasoconstriction in resting efferent arterioles, but a stronger efferent arteriolar constriction following treatment with L-NNA. We conclude that NO inhibits voltage-gated L- and T-type Ca2+ channel activity either directly or indirectly in both afferent and efferent arterioles. The ability of L-type Ca2+ channel blockade to prevent the efferent arteriolar constriction elicited by L-NNA contrasts with studies showing a lack of effects of L-type Ca2+ channel blockade on resting and SNAP-treated L-NNA efferent arterioles and the difference in responses to high KCl between resting and L-NNA-constricted efferent arterioles suggests that endogenous NO normally suppresses L-type Ca2+ channel expression or activity in efferent arterioles and that NOS inhibition leads to recruitment or activation of these usually quiescent L-type Ca2+ channels in efferent arterioles. From a pathophysiological perspective, endothelial dysfunction may lead to enhanced expression of L-type Ca2+ channels in postglomerular arterioles and increase postglomerular resistance exacerbating glomerular hypertension and renal damage, as has been shown for L-NAME-treated SHR (30). Thus L-NNA-mediated arterial vasoconstriction may involve activation of T- and L-type Ca2+ channels in both afferent and efferent arterioles. L-type Ca2+ channels may act cooperatively with T-type channels to play important roles in mediating Ca2+ entry responsible for L-NNA-induced efferent and afferent arteriolar constriction.

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