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

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Feng, Ming-Guo, and L. Gabriel Navar. Nitric oxide synthase inhibition activates L- and T-type Ca$^{2+}$ channels in afferent and efferent arterioles. Am J Physiol Renal Physiol 290: F873–F879, 2006.—Previous studies have shown that L-type Ca$^{2+}$ channel (LCC) blockers primarily dilate resting and ANG II-constricted afferent arterioles (AA), but do not influence either resting or ANG II-constricted efferent arterioles (EA). In contrast, blockade of T-type Ca$^{2+}$ channels (TCC) dilate EA and prevent ANG II-mediated efferent constriction. This study determined the role of LCC and TCC in mediating the AA and EA constriction following inhibition of nitric oxide synthase (NOS) and tested the hypothesis that inhibition of NOS increases the influence of LCC on EA. With the use of an isolated blood-perfused rat juxtamedullary nephron preparation, single AA or EA were visualized and superfused with a NOS inhibitor, N-nitro-l-arginine (l-NNA), with or without concomitant treatment with an LCC blocker, diltiazem, or a TCC blocker, pimozide. In response to l-NNA (1, 10, and 100 μmol/l), AA and EA diameters decreased significantly by 6.0 ± 0.3, 13.7 ± 1.7, and 19.9 ± 1.4%, and by 6.2 ± 0.5, 13.3 ± 1.1, and 19.0 ± 1.9%, respectively. During TCC blockade with pimozide (10 μmol/l), l-NNA did not significantly constrict afferent (0.9 ± 0.6, 15 ± 0.5, and 17 ± 0.5%) or efferent (0.4 ± 0.1, 2.1 ± 0.7, and 2.5 ± 1.0%) arterioles. In contrast to the responses with other vasoconstrictors, the l-NNA-induced constriction of EA, as well as AA, was reversed by diltiazem (10 μmol/l). The effects were overlapping as pimozide superimposed on diltiazem did not elicit further dilation. When the effects of l-NNA were reversed by superfusion with an NO donor, SNAP (10 μmol/l), diltiazem did not cause significant efferent dilation. As a further test of LCC activity, 55 mmol/l KCl, which depolarizes and constricts AA, was applied to AA and to EA. The difference in responses to high KCl between resting and l-NNA-constricted AA and the ability 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.

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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 vasoconstriction, 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$^{2+}$ 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 T-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$^{G}$-nitro-l-arginine methyl ester (l-NNAME), 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 efferent arteriolar diameters in normotensive (20, 32) and ANG II-infused hypertensive rat kidneys (19). Administration of mibebradil, 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-NNAME in drinking water for 3 wk to SHR (30). It was also demonstrated that pimozide and mibebradil, potent T-type Ca$^{2+}$ channel blockers (1, 2, 7, 10, 34, 36), vasodilate both afferent and efferent arterioles in the isolated blood-perfused juxtamedullary nephron preparation of Sprague-Dawley rats (10), indicating that T-type Ca$^{2+}$ channels are functionally expressed in both renal microcirculation; calcium channel blockers; nitric oxide synthase inhibitors; diltiazem; pimozide

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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 arteriole 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 Ca\(^{2+}\) channels (1). One index of selectivity is that diltiazem prevented the high KCl-induced afferent arteriolar constriction which is due to direct opening of L-type Ca\(^{2+}\) 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 assessed 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 pentoxybarbital 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. The buffy 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 33%. 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 that were 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 MFJ-1452; MFJ 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 imageshearing 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 superfusate 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 efferent constriction was reversed by superfusion with a NO donor, S-nitroso-N-acetylpencillamine (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 isotonc 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 II, 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 control condition.
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 mmol/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 mmol/l)-containing solutions elicited further strong constriction of both resting and L-NNa-preconstricted efferent arterioles, with 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 58.6 ± 5.9% (n = 5, P < 0.01), respectively. The effects of high KCl and NE on resting and L-NNa-preconstricted efferent arteriolar diameters are shown in Fig. 5. The average efferent arteriole diameter of 20.0 ± 0.5 μm was decreased by 10.2 ± 0.3 μm (P < 0.01) for an increase in efferent arteriole diameter of 20.0 ± 0.5 μm (n = 5, 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 μmol/l), diltiazem caused a modest or nonsignificant dilatation, with average diameter increasing by 3.8 ± 0.8% (n = 5, P < 0.05). In contrast, superfusion with solutions containing pimozide (10 μmol/l) caused significant dilatation of resting efferent arterioles, with average diameter increasing by 23.5 ± 6.6 μm, 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 dilatation 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 L-NNa and pimozide on resting and L-NNa-preconstricted efferent arterioles. 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 μmol/l), diltiazem only caused a modest or nonsignificant dilatation, with average diameter increasing by 3.8 ± 0.8% (n = 5, P < 0.05). In contrast, superfusion with solutions containing pimozide (10 μmol/l) caused significant dilatation of resting efferent arterioles, with average diameter increasing from 19.4 ± 0.2 to 23.5 ± 6.6 μm, 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 dilatation 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.

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 μmol/l), diltiazem only caused a modest or nonsignificant dilatation, with average diameter increasing by 3.8 ± 0.8% (n = 5, P < 0.05). In contrast, superfusion with solutions containing pimozide (10 μmol/l) caused significant dilatation of resting efferent arterioles, with average diameter increasing from 19.4 ± 0.2 to 23.5 ± 6.6 μm, 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 dilatation 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.

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 μmol/l) constricted efferent arterioles, and the vasoconstriction was reversed by adding SNAP (10 μmol/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 arteriole diameter of 21.5 ± 2.3%. Adding diltiazem (10 μmol/l) to the SNAP-containing solutions only caused a slight and nonsignificant increase in efferent arteriole 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.

Fig. 3. Efferent arteriolar response to L-NNa followed by treatment with S-nitroso-N-acetylpenicillamine (SNAP) and diltiazem L-NNa (100 μmol/l) caused constriction of efferent arterioles (n = 5), which was reversed by adding SNAP (10 μmol/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 arteriole diameter of 21.5 ± 2.3%. Adding diltiazem (10 μmol/l), in the presence of SNAP, caused only a slight and nonsignificant increase in efferent arteriole diameter, changing from 19.7 ± 0.7 to 20.5 ± 0.8 μm, (n = 5, P > 0.05). Values are means ± SE. *P < 0.05, **P < 0.01 compared with control condition. #P < 0.05, ###P < 0.01 compared with L-NNa alone.

Fig. 4. Comparison of the effects of diltiazem and pimozide on resting and L-NNa-preconstricted efferent arteriolar diameters. Diltiazem significantly inhibited the L-NNa-induced constriction in L-NNa-preconstricted efferent arterioles (n = 6), but not in resting (n = 6) and SNAP-treated L-NNa-preconstricted arterioles (n = 5). In contrast, pimozide markedly inhibited the L-NNa-induced constriction in resting efferent arterioles (n = 5). Values are means ± SE. **P < 0.01 compared with baseline.
regulation of efferent arterioles. However, we acknowledge that these conclusions are based on the results obtained using only one L-type Ca$^{2+}$ channel blocker and one T-type Ca$^{2+}$ 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$^{2+}$ channel blockers are needed to provide further support to these conclusions.

The mechanisms linking NO signaling pathways and voltage-gated Ca$^{2+}$ 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$^{2+}$ release stimulated by inositol 1,4,5-triphosphate (IP$_3$) in VSM (24, 26, 38). NO also influences voltage-gated Ca$^{2+}$ channel activity. In cultured VSM cells, sodium nitroprusside (SNP), a vasodilator that provides NO, reduced [Ca$^{2+}$], in cells in which [Ca$^{2+}$] 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$^{2+}$], by inhibition of Ca$^{2+}$ entry. In VSM cells of rat tail artery, Ca$^{2+}$ entry through voltage-operated calcium channels is more sensitive to NO compared with receptor-operated calcium channels or intracellular Ca$^{2+}$ 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$^{2+}$ from the sarcoplasmic reticulum, indicating that the response was mediated by Ca$^{2+}$ influx rather than by release from intracellular stores (16). NO has also been reported to inhibit L-type Ca$^{2+}$ 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$^{2+}$-activated K$^+$ channels (KCa) (37) and L-type Ca$^{2+}$ 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 NOS would be expected to reduce the cGMP-mediated inhibition of IP$_3$, allowing increased IP$_3$-mediated release of intracellular Ca$^{2+}$. This effect, coupled with blockade of potassium channels, might reduce membrane potential sufficiently to activate T-type Ca$^{2+}$ channels which, in turn, would reduce membrane potential and further activate L-type Ca$^{2+}$ channels, thus increasing entry of Ca$^{2+}$, leading to afferent and efferent arteriolar vasoconstriction. It is likely that there is a cooperativity between LVA Ca$^{2+}$ channels and HVA Ca$^{2+}$ channels to control the influx of Ca$^{2+}$ ions in afferent and efferent arteriolar VSM cells under conditions of NOS inhibition (10, 18).

A novel aspect of the present findings is that L-type Ca$^{2+}$ channel blockade prevented the efferent arteriolar vasoconstriction elicited by L-NNA. The effects of L-type Ca$^{2+}$ 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$^{2+}$ 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 postglomerular 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 constriction elicited by 55 mM KCl, we found that 55 mM KCl caused no signficant dilatation of l-NNA-preconstricted efferent arterioles and normally exert only modest effects on postglomerular arterioles. These results further support the notion that L-type Ca²⁺ channels in efferent arterioles elicited by high KCl to reverse the effects of l-NNA, we performed NO donor studies. We found that after restoration of NO levels by superfusing with a NO donor, SNAP, diltiazem did not cause significant dilatation of l-NNA-treated efferent arterioles indicating that NO inhibits L-type Ca²⁺ channel in efferent arterioles. The results support the notion that NOS inhibition recruits L-type channels in efferent arterioles. The difference in response to high KCl between resting and l-NNA-preconstricted efferent arterioles and the ability of diltiazem to block efferent arteriolar vasoconstriction 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 (11), suggesting a recruitment of L-type Ca²⁺ channels in efferent arterioles specifically in response to NOS inhibition.

The present study also shows that adding pimozide did not significantly augment the dilatation of afferent and efferent arterioles elicited by diltiazem. In afferent arterioles and under conditions of increased expression or activation of L-type Ca²⁺ channels in efferent arterioles, blockade of the HVA T-type channels may result in membrane hyperpolarization, which may shift the membrane potential out of the LVA T-type window-current range of voltages and lead to inactivation of the LVA T-type Ca²⁺ channels. It is also likely that in afferent and efferent arterioles, the l-NNA-induced decrease in NO activity allows enough depolarization for the smooth muscle cell to cause activation of T-type Ca²⁺ channels which, in turn, elicits sufficient depolarization to activate L-type Ca²⁺ channels. Blockade of the LVA T-type channels may result in membrane hyperpolarization and lead to inactivation of the HVA L-type Ca²⁺ channels (10, 18). Thus L-type Ca²⁺ channels may act cooperatively with T-type channels to mediate Ca²⁺ entry responsible for NOS inhibition-mediated efferent and afferent arteriole vasoconstriction.

In summary, the present results demonstrate that l-NNA caused dose-dependent vasoconstriction of afferent and efferent arterioles, which was markedly diminished by the T-type Ca²⁺ channel blocker, pimozide, and L-type Ca²⁺ channel blocker, diltiazem. While diltiazem had no significant effect on resting and SNAP-treated l-NNA-preconstricted efferent arterioles, it did reverse the vasoconstriction elicited by l-NNA. 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 NO inhibition leads to recruitment or activation of these usually quiescent L-type Ca²⁺ channels in efferent arterioles.

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