NO\textsuperscript{−} Activates Soluble Guanylate Cyclase and K\textsubscript{v} Channels to Vasodilate Resistance Arteries

Jennifer C. Irvine, Joanne L. Favaloro, Barbara K. Kemp-Harper

Abstract—Nitric oxide (NO) plays an important role in the control of vascular tone. Traditionally, its vasorelaxant activity has been attributed to the free radical form of NO (NO\textsuperscript{*}), yet the reduced form of NO (NO\textsuperscript{−}) is also produced endogenously and is a potent vasodilator of large conduit arteries. The effects of NO\textsuperscript{−} in the resistance vasculature remain unknown. This study examines the activity of NO\textsuperscript{−} in rat small isolated mesenteric resistance-like arteries and characterizes its mechanism(s) of action. With the use of standard myographic techniques, the vasorelaxant properties of NO\textsuperscript{*} (NO gas solution), NO\textsuperscript{−} (Angeli’s salt), and the NO donor sodium nitroprusside were compared. Relaxation responses to Angeli’s salt (pEC\textsubscript{50}=7.51±0.13, R\textsubscript{max}=95.5±1.5%) were unchanged in the presence of carboxy-PTIO (NO\textsuperscript{*} scavenger) but those to NO\textsuperscript{−} and sodium nitroprusside were inhibited. L-Cysteine (NO\textsuperscript{−} scavenger) decreased the sensitivity to Angeli’s salt (P<0.01) and sodium nitroprusside (P<0.01) but not to NO\textsuperscript{*}. The soluble guanylate cyclase inhibitor ODQ (3 and 10 μmol/L) concentration-dependently inhibited relaxation responses to Angeli’s salt (41.0±6.0% versus control 93.4±1.9% at 10 μmol/L). The voltage-dependent K\textsuperscript{+} channel inhibitor 4-aminopyridine (1 mmol/L) caused a 9-fold (P<0.01) decrease in sensitivity to Angeli’s salt, whereas glibenclamide, iberiotoxin, charybdotoxin, and apamin were without effect. In combination, ODQ and 4-aminopyridine abolished the response to Angeli’s salt. In conclusion, NO\textsuperscript{−} functions as a potent vasodilator of resistance arteries, mediating its response independently of NO\textsuperscript{*} and through the activation of soluble guanylate cyclase and voltage-dependent K\textsuperscript{+} channels. NO\textsuperscript{−} donors may represent a novel class of nitrovasodilator relevant for the treatment of cardiovascular disorders such as angina. (Hypertension. 2003;41:1301-1307.)

Key Words: nitric oxide ■ nitroxy anion ■ potassium channels ■ vasorelaxation ■ vasculature ■ mesenteric arteries

The endogenous production of nitric oxide (NO) plays an important role in the control of vascular tone and blood pressure.\textsuperscript{1} To date, most of the vascular effects of NO have been attributed to the free radical form of NO (NO\textsuperscript{*}), which mediates vascular smooth muscle relaxation predominantly through the activation of soluble guanylate cyclase and subsequent accumulation of cGMP.\textsuperscript{1} NO, however, can also exist in the oxidized state as the nitrosoum cation (NO\textsuperscript{+}) and in the reduced state as the nitroxy anion (NO\textsuperscript{−}). Little attention has been afforded to the biological activity of these alternative redox forms of NO, yet recent findings that the nitroxy anion is produced endogenously\textsuperscript{2,3} and displays relaxant activity within the vasculature\textsuperscript{4,5} highlights a possible physiological role for this nitrogen oxide species.

NO\textsuperscript{−} can be generated directly from the enzymatic activity of NO synthase (NOS)\textsuperscript{2,3} such that NOS-catalyzed oxidation of l-arginine results in the production of NO\textsuperscript{−}, which is further oxidized to NO\textsuperscript{*} by superoxide dismutase. In addition, NO\textsuperscript{−} can also be formed during oxidation of the decoupled NOS product N-hydroxy-l-arginine,\textsuperscript{6,7} from NOS in the absence of tetrahydrobiopterin,\textsuperscript{8} after the decomposition of S-nitrosothiols\textsuperscript{9,10} and peroxynitrite\textsuperscript{11} and from the reduction of NO\textsuperscript{*} by mitochondrial cytchrome c.\textsuperscript{12}

The biological activity of NO\textsuperscript{−} can be studied by using NO\textsuperscript{*} donors such as Angeli’s salt. Of particular interest is the identification of NO\textsuperscript{−} as a potent vasodilator, mediating relaxation of large isolated conduit arteries,\textsuperscript{4,5,13} exerting dilator activity in the intact pulmonary vascular bed,\textsuperscript{14} and decreasing mean arterial blood pressure in the anesthetized rabbit.\textsuperscript{15} The relaxation response to NO\textsuperscript{−} in large arteries is accompanied by an increase in cyclic GMP\textsuperscript{13} and attenuated by the soluble guanylate cyclase (sGC) inhibitor ODQ.\textsuperscript{4,5}

The finding that NO\textsuperscript{−} can be formed endogenously and mediates vascular smooth muscle relaxation raises the exciting possibility that NO\textsuperscript{−} may contribute to vasodilator responses previously attributed to NO\textsuperscript{*}. Indeed, it has recently been suggested that the relaxation attributed to endothelium-derived relaxing factor in both rat\textsuperscript{4} and mouse\textsuperscript{5} aortae is mediated in part by NO\textsuperscript{−}. These studies emphasize the potential physiological importance of NO\textsuperscript{−}.

The full potential of NO\textsuperscript{−} as both an endogenous and exogenous regulator of vascular tone, however, will not be
realized until its effects within the resistance vasculature are studied. Currently, the role of NO⁻ in resistance vessels is unknown; thus, this study aimed to investigate the mechanisms whereby NO⁻ mediates vasorelaxation of small mesenteric resistance arteries.

Methods

This study was approved by the Pharmacology Animal Ethics Committee, Monash University, Australia.

Isolation and Study of Mesenteric Arteries

Male Wistar-Kyoto rats were killed by means of stunning and exsanguination. Small mesenteric arteries (third-order branch of the superior mesenteric artery) were isolated, cut into 2-mm lengths, and mounted in isometric myographs. Vessels were maintained in physiological Krebs solution at 37°C and continuously bubbled with carbogen (95% O₂, 5% CO₂). Data were captured through the use of the CVMS data acquisition system (World Precision Instruments). After a 30-minute equilibration period, vessel diameters were normalized to an equivalent transmural pressure of 100 mm Hg.³

Functional Experiments

Thirty minutes after normalization, vessels were maximally contracted with a K⁺-depolarizing solution (KPSS). Responses to vasorelaxants were then examined in arteries precontracted to ~50% KPSS with methoxamine (0.1 to 5 μmol/L). Cumulative concentration-response curves to either the NO⁻ donor Angeli’s salt (0.1 mmol/L to 100 μmol/L), sodium nitroprusside (SNP; 0.1 mmol/L to 100 μmol/L), NO⁺ (1 mmol/L to 30 μmol/L; aqueous solution of NO gas), or the cGMP analogue 8-pCPT-cGMP (0.1 to 100 μmol/L) were constructed. Only one concentration-response curve to any vasodilator was obtained for each vessel segment.

Initially, relaxation responses to Angeli’s salt were examined in Krebs solution with and without the copper chelator EDTA. Subsequent experiments were conducted in Krebs solution containing EDTA. Responses to Angeli’s salt, SNP, and NO⁺ were obtained in the absence and presence of the NO⁻ scavenger l-cysteine (3 mmol/L) and the NO⁺ scavenger carboxy-PTIO (200 μmol/L), which were added 3 and 15 minutes before precontraction with methoxamine, respectively.

In the presence of carboxy-PTIO (200 μmol/L), responses to Angeli’s salt were also examined in the absence or presence of either (1) ODQ (3 and 10 μmol/L), (2) the K⁺-depolarizing solution (30 mmol/L K⁺), (3) ODQ (3 or 10 μmol/L) + 30 mmol/L K⁺, (4) iberiotoxin (100 μmol/L), (5) charybdotoxin (100 μmol/L), (6) apamin (100 μmol/L), (7) 4-aminopyridine (1 mmol/L), (8) glibenclamide (10 μmol/L), and (9) 4-aminopyridine (1 mmol/L) + ODQ (10 μmol/L). Inhibitors were added 30 minutes before precontraction with methoxamine.

Responses to 8-pCPT-cGMP were also examined in the absence or presence of 4-aminopyridine (1 mmol/L).

NO-Sensitive Electrode Experiments

NO⁺ release was measured with an ISO-NOP nitric oxide sensor electrode (World Precision Instruments). The electrode was calibrated by adding known volumes of a standard KNO₂ solution to a solution of KI (0.1 mmol/L) and H₂SO₄ (0.1 mol/L) at 37°C. Subsequently, the NO⁺ electrode was immersed in a closed 10-mL vial containing carbogenated Krebs solution (37°C) and exposed to increasing concentrations of Angeli’s salt (1 mmol/L to 100 μmol/L), in the absence and presence of carboxy-PTIO (200 μmol/L). In some experiments, EDTA (0.026 mmol/L) was included in the Krebs solution.

Data Analysis

Relaxation responses are expressed as a percentage reversal of the methoxamine precontraction. Individual relaxation curves were fitted to a sigmoidal logistic equation (Graphpad Prism 3.0) and pEC₅₀ values (concentration of agonist causing a 50% relaxation) calculated and expressed as −log M. Differences between mean pEC₅₀ and maximum relaxation (Rmax) values were tested by using either a Student unpaired t test or 1-way ANOVA (Graphpad Prism 3.0). Concentration-response curves were compared by means of a 2-way ANOVA (Sigma Stat 1.0). Results are expressed as mean±SEM, and statistical significance was accepted at a level of P<0.05.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Relaxation Responses to Angeli’s Salt and NO⁺ Generation

The NO⁺ donor Angeli’s salt caused concentration-dependent relaxations (pEC₅₀=7.77±0.07, Rmax=96.6±0.7%) in endothelium-intact small mesenteric arteries (D₁₀₀=375±4 μm; Figure 1A). Removal of the endothelium did not alter the response to Angeli’s salt (data not shown). In the Krebs solution, a NO⁺ signal was detected at concentrations of Angeli’s salt ≥0.3 μmol/L with 1097±139 nmol/L NO⁺ generated in response to 100 μmol/L Angeli’s salt. The NO⁺ scavenger carboxy-PTIO (200 μmol/L) decreased the NO⁺ signal generated from Angeli’s salt (P<0.05) and caused a significant rightward shift in the relaxation-response curve (pEC₅₀=7.02±0.16; P<0.01) (Figure 1A).
The inclusion of the Cu²⁺-chelator EDTA (0.026 mmol/L) in the Krebs solution markedly reduced the NO⁺ signal generated in response to Angeli’s salt (477±127 mmol/L at 100 μmol/L Angeli’s salt; Figure 1B). Furthermore, in the presence of EDTA, neither Angeli’s salt–mediated relaxations (pEC₅₀=7.51±0.13, R_max=95.5±1.5%) nor the production of NO⁺ was affected by carboxy-PTIO (Figure 1B). The vehicle for Angeli’s salt (0.01 mol/L NaOH) did not induce relaxation or generate a NO⁺ signal (data not shown). All subsequent responses to Angeli’s salt were examined in the presence of EDTA (0.026 mmol/L) and carboxy-PTIO (200 μmol/L), and concentrations of Angeli’s salt >10 μmol/L were not used.

The second decomposition product of Angeli’s salt, nitrite, was a 15 000-fold less potent vasodilator (NaNO₂: pEC₅₀=3.81±0.17, R_max=87.0±4.7%; n=5) of small mesenteric arteries compared with NO⁺.

**Effect of Carboxy-PTIO and L-Cysteine on Responses to Angeli’s Salt, NO Gas, and SNP**

As indicated previously, the NO⁺ scavenger carboxy-PTIO had no effect on relaxation responses to Angeli’s salt (Figure 2A). In contrast, carboxy-PTIO (200 μmol/L) decreased the sensitivity to NO gas (Figure 2B) and SNP (Figure 2C) 10-fold (P<0.05) and 21-fold (P<0.01), respectively. Carboxy-PTIO also attenuated the relaxation response to 30 μmol/L NO gas (R_max=68.1±7.0% versus control R_max=90.5±2.4%; P<0.05)

The NO⁺ scavenger L-cysteine (3 mmol/L) caused a significant rightward shift in the concentration-response curve to Angeli’s salt (pEC₅₀=6.09±0.13, R_max=87.1±2.3%; P<0.01) compared with control (pEC₅₀=7.58±0.10, R_max=94.2±0.8%; Figure 2A) but did not alter the maximum response. L-Cysteine failed to alter the response to NO gas (Figure 2B) yet caused a significant decrease in sensitivity to SNP (pEC₅₀=6.36±0.20 versus control pEC₅₀=8.26±0.06; P<0.01, Figure 2C).

**Effect of ODQ and High K⁺ on Responses to Angeli’s Salt**

The sGC inhibitor ODQ, at concentrations of 3 and 10 μmol/L, caused a marked and concentration-dependent attenuation (P<0.05) of the relaxation-response curve to Angeli’s salt (Figure 3A). This was accompanied by a significant (P<0.001) decrease in the response to 10 μmol/L Angeli’s salt (3 μmol/L ODQ: R_max=78.0±3.7%; 10 μmol/L ODQ: R_max=41.0±6.0%) compared with control (R_max=93.4±1.9%). ODQ at 10 μmol/L impaired Angeli’s salt–mediated relaxation to a significantly greater extent than did 3 μmol/L ODQ (P<0.05).

Raising the extracellular concentration of K⁺ to 30 mmol/L also caused a significant 20-fold (P<0.001) decrease in sensitivity to Angeli’s salt and reduced the relaxation response to 10 μmol/L Angeli’s salt (R_max=83.2±3.3%; P<0.01) compared with control (pEC₅₀=7.67±0.22, R_max=95.9±0.6%; Figure 3B). ODQ at concentrations of either 3 or 10 μmol/L in combination with 30 mmol/L K⁺ virtually abolished the response to Angeli’s salt (Figure 3C).

![Figure 2](image-url)  
**Figure 2.** Concentration-response curves to A, Angeli’s salt (NO⁺); B, authentic NO gas (NO); and C, SNP (NO⁺) in rat small mesenteric arteries in the absence (○, n=5 to 12) and presence of carboxy-PTIO (200 μmol/L; ●, n=4 to 7) and l-cysteine (3 mmol/L; *, n=4 to 7). EDTA (0.026 mmol/L) was present throughout. Values are expressed as percentage reversal of level of precontraction and given as mean±SEM, where n=number of vessel segments. *P<0.05, **P<0.01 for pEC₅₀ value vs untreated control (1-way ANOVA, Dunnett modified t test). Single or double asterisk indicates P<0.05 for response at 30 μmol/L NO⁺ vs untreated control (1-way ANOVA, Dunnett modified t test).

**Effect of Specific K⁺ Channel Inhibitors on Responses to Angeli’s Salt**

The relaxation response to Angeli’s salt (pEC₅₀=7.75±0.16, R_max=93.2±2.2%) was unchanged in the presence of the K⁺ channel inhibitors glibenclamide (10 μmol/L), apamin (100 nM), iberiotoxin (100 nM), and charybdotoxin (100 nM). In contrast, a significant decrease in sensitivity to Angeli’s salt was seen after treatment with the voltage-sensitive K⁺ channel (Kᵥ) inhibitor 4-aminoypyridine (1 mmol/L: pEC₅₀=6.78±0.06, R_max=93.6±2.7%; P<0.01, Figure 4A). ODQ (10 μmol/L) and 4-aminoypyridine...
(1 mmol/L) in combination abolished the vasorelaxant response to Angeli’s salt (Figure 4B).

The relaxation response to the cGMP analogue 8-pCPT-cGMP (pEC50 = 4.82 ± 0.09, Rmax = 80.4 ± 1.5%) was unchanged in the presence of high K⁺ or 4-aminopyridine (Figure 5).

Discussion

This study has demonstrated for the first time an ability of NO⁻, the one electron reduction product of NO⁺, to function as a potent vasodilator of rat small isolated mesenteric resistance–like arteries. Furthermore, NO⁻ mediates vasorelaxation in these vessels independently of NO⁺ and through the activation of sGC and K⁺ channels.

Given that NO⁻ can undergo oxidation to NO⁺, both extracellularly and intracellularly, we initially elucidated the possible contribution of NO⁺ to responses to the NO⁻ donor Angeli’s salt. This was achieved through the use of two experimental approaches. First, a NO⁺-sensitive electrode was used to detect NO⁺ generation after decomposition of Angeli’s salt in Krebs solution. Second, several pharmacological agents were used to identify the species of NO mediating relaxation responses to Angeli’s salt.

Under our experimental conditions, it was apparent that NO⁻ donated from Angeli’s salt, was oxidized in part to NO⁺. Thus high concentrations of Angeli’s salt (≥1 mmol/L) decomposed in Krebs solution to generate a substantial...
amount of NO*, as detected with the NO*-sensitive electrode. Furthermore, the NO* scavenger carboxy-PTIO caused a rightward shift in the relaxation response curve to Angeli’s salt, reflecting the loss of NO* formed from Angeli’s salt and the continuing vasorelaxation caused by NO*.

Given that copper can catalyze the oxidation of NO* to NO**,19,20 the effect of the Cu2+-chelator EDTA (0.026 mmol/L) on the decomposition of Angeli’s salt was studied. Indeed, in the presence of EDTA, NO* generation in the Krebs solution was markedly reduced, such that NO* was not detected at concentrations of Angeli’s salt of <10 μmol/L. These findings are in agreement with those of other investigators3,20,26 and clearly indicate that the presence of Cu2+ ions in the experimental media (Krebs solution) leads to the partial oxidation of NO* donated from Angeli’s salt, to NO*. In further support of this observation, Angeli’s salt-mediated relaxation was unaffected by carboxy-PTIO in the presence of EDTA.

To further distinguish between the redox forms of NO that may contribute to the relaxant activity of Angeli’s salt, a number of pharmacological agents were used. Carboxy-PTIO and l-cysteine have been shown to distinguish between NO* and NO−, respectively. Thus, the free radical scavenger carboxy-PTIO inactivates NO* but not NO−,4,5,23 and the thiol l-cysteine, at millimolar concentrations, attenuates the actions of NO− yet enhances and prolongs the activity of NO*.4,5,24

Accordingly, the NO* scavenger carboxy-PTIO attenuated the relaxation response to NO gas solution but did not alter that to Angeli’s salt. Conversely, l-cysteine inhibited Angeli’s salt–mediated relaxation but not that to NO gas. Similar findings have been made in the rat isolated anococcygeus23 and aorta4 and mouse aorta.25 Interestingly, we found that vasorelaxation to the NO donor SNP in rat small mesenteric arteries was impaired by both carboxy-PTIO and l-cysteine. This was also the observation of Wanstall and colleagues5 in the mouse aorta, and, together, these findings suggest that SNP donates both NO* and NO−.

It appears, therefore, that under the experimental conditions used in the current study, namely, the inclusion of EDTA in the Krebs solution, NO− donated from Angeli’s salt (<10 μmol/L) is not oxidized to NO* extracellularly (eg, before entry into the vascular smooth muscle). These findings are similar to those in the rat isolated anococcygeus,23 yet contrast studies in the rat isolated aorta,20 where, even in the presence of EDTA, carboxy-PTIO markedly attenuated Angeli’s salt–mediated relaxation. Such a discrepancy may reflect differences in the oxidative environment between large conduit and small resistance arteries. Specifically, the aorta may have cellular components at the level of the smooth muscle that readily convert NO− to NO*, and/or mesenteric resistance arteries may contain an antioxidant that prevents the oxidation of NO− to NO*. Nevertheless, our NO* electrode studies coupled with functional experiments established the optimal conditions whereby to investigate the role of NO− in the resistance vasculature. Namely, all subsequent experiments were conducted in Krebs solution containing EDTA (0.026 mmol/L) and carboxy-PTIO (200 μmol/L), and concentrations of Angeli’s salt >10 μmol/L were not used.

Although Angeli’s salt decomposes to produce both NO− and nitrite anions (NO2−), it is unlikely that the relaxation to Angeli’s salt in mesenteric resistance arteries could be attributed to NO2−, as NO2− was a 15 000-fold less potent vasodilator compared with Angeli’s salt in this vascular preparation.

We next sought to characterize the mechanism(s) whereby NO− mediates vasorelaxation of rat small mesenteric arteries. In agreement with previous studies in vascular4,5 and nonvascular preparations,23,24 the sGC inhibitor ODQ markedly attenuated the relaxation response to Angeli’s salt. Thus, responses to NO− appear to be mediated predominantly through the activation of the sGC/cGMP pathway. Given that Dierks and Burstyn21 have suggested that NO* is the only redox form of NO that can directly activate sGC, our findings, together with those of other investigators,4,5 suggest that NO− may be oxidized to NO*. If such a conversion of NO− to NO* were to take place, it must occur intracellularly, as we have clearly demonstrated that NO− is not oxidized in the extracellular media. Indeed, a number of candidates have been proposed as intracellular oxidants including superoxide dismutase,3,18 flavins,26 copper-containing enzymes,19,22,24 quinones,27 and cytochrome P450.21 It remains to be determined if such oxidants play a role in the conversion of NO− to NO* in the resistance vasculature.

We cannot exclude the possibility, however, that NO− itself can directly activate sGC, particularly given that previous studies have shown that relaxant responses to NO− are more susceptible to inhibition by ODQ than those to NO*.4,5,23 If NO− were to directly activate sGC, it would presumably have to traverse the cell membrane. Yet, the negatively charged nature of this NO species may oppose such an action. It is tempting to speculate, therefore, that anion channels may facilitate the movement of NO− across cellular membranes, although no studies to date have investigated this hypothesis.

Interestingly, we found that ODQ, at a concentration of 10 μmol/L, failed to abolish the relaxation to Angeli’s salt in rat small mesenteric arteries. Similarly, responses to Angeli’s salt in the mouse isolated aorta have been shown to be resistant, in part, to ODQ.5 It is unlikely that the concentration of ODQ used (10 μmol/L) was insufficient to inhibit sGC.
completed, given that a concentration of 10 to 60 nmol/L is required to achieve half-maximal inhibition of sGC, and ODQ at a concentration of 3 μmol/L has been shown to abolish cGMP production in response to a NO donor. Rather the inability of ODQ to abolish responses to Angeli’s salt suggests that NO—may also mediate vascular smooth muscle relaxation in resistance arteries through a cGMP-independent mechanism.

Indeed, our finding that raising the extracellular concentration of K+ (30 mmol/L) attenuated the relaxation to Angeli’s salt and furthermore abolished the ODQ-resistant response suggested that NO—mediates relaxation of rat small mesenteric arteries in part through the activation of K+ channels and subsequent smooth muscle hyperpolarization. Specifically, we identified an ability of NO— to activate a voltage-gated K+ (Kv) channel, as relaxation responses to Angeli’s salt were impaired by the Kv channel inhibitor 4-aminopyridine. Neither ATP-sensitive (KATP) nor Ca2+-activated (KCa) K+ channels appeared to be activated by NO—, as glibenclamide, iberiotoxin, charybdotoxin, and apamin were without effect.

In the absence of patch-clamp studies, we can only suggest that the inhibitory effect of 4-aminopyridine on Angeli’s salt–mediated relaxation is indicative of the activation of Kv channels by NO—. It is certainly feasible that NO— activates a Kv channel in resistance arteries because the Kv channel subunits, Kv1.2, Kv1.3, and Kv1.5 are expressed in rat mesenteric artery smooth muscle cells. Furthermore, preliminary electrophysiological experiments in our laboratory have demonstrated an ability of Angeli’s salt to hyperpolarize rat small mesenteric arteries, depolarized with phenylephrine, through a 4-aminopyridine–sensitive mechanism (Favaloro and Kemp-Harper, unpublished data). In addition, our findings concur with a previous study in a nonvascular preparation, the sheep isolated urethra, where relaxation responses to Angeli’s salt were found to be impaired in part by 4-aminopyridine. Our study, together with that of Costa and colleagues, suggests an ability of NO— to activate Kv channels.

Whether NO— activates Kv channels directly or through a cGMP-dependent mechanism remains to be determined. However, our findings that a component of the relaxation response to Angeli’s salt was resistant to ODQ and vasorelaxation to the cell-permeable cGMP analogue 8-pCPT-cGMP was unchanged in the presence of 4-aminopyridine suggests that NO— may activate Kv channels, at least in part, independently of cGMP. Indeed, the free radical form of NO— has been found to directly activate Kv channels of the KCa subtype. In contrast, Costa and colleagues have proposed that NO— activates Kv channels in sheep urethral smooth muscle through a cGMP-dependent mechanism. Although these investigators also found that the action of 8-bromo-cGMP was not affected by 4-aminopyridine, their observation that the response to a single concentration of Angeli’s salt (0.1 mmol/L) was abolished by ODQ and that 4-aminopyridine failed to antagonize the actions of Angeli’s salt in the presence of the phosphodiesterase inhibitor zaprinast (a state of elevated cGMP) led them to conclude that NO— activated K+ channels in a cGMP-dependent manner. Indeed, cGMP itself can activate K+ channels, albeit of the KCa subtype.

Nevertheless, the possibility that NO— activates Kv channels in part through the activation of sGC cannot be excluded by the findings of the current study. Given that endogenously and exogenously generated cGMP may mediate relaxation through different mechanisms, a lack of effect of 4-aminopyridine on the response to 8-pCPT-cGMP does not necessarily preclude an ability of cGMP to activate Kv channels. Future studies that use electrophysiological techniques to record changes in membrane potential will allow us to elucidate the exact mechanism whereby NO— activates Kv channels and hyperpolarizes the vascular smooth muscle.

Interestingly, in rat small mesenteric arteries, NO• at high concentrations activates a charybdotoxin-sensitive K+ channel (KCa) through a cGMP-independent mechanism. Taken together with our findings, it may be suggested that NO• and NO— can mediate vasorelaxation of resistance arteries through distinct mechanisms, namely, the activation of KCa and Kv channels, respectively. These findings are of particular importance in light of the recent reports that the activity of endothelium-derived relaxing factor in large conduit arteries more closely resembles the properties of NO— rather than NO•. Indeed, future studies examining the contribution of NO— to endothelium-dependent relaxation in the resistance vasculature will further clarify the importance of endogenously generated NO— in the regulation of vascular tone.

Perspectives

In conclusion, this study has provided evidence to suggest an ability of NO— to mediate relaxation of resistance-like arteries through the activation of soluble guanylate cyclase and Kv channels. Furthermore, it appears that this activation of Kv channels by NO— is, at least in part, independently of cGMP. These results suggest that NO— and NO• may activate distinct Kv channels to mediate their response. From a clinical viewpoint, NO— donors may represent a novel class of nitrovasodilator for the treatment of cardiovascular disease. Thus, the distinct mechanism of action of NO— may facilitate the use of NO— donors in the treatment of disorders such as angina, in which the current use of traditional nitrovasodilators is often limited due to the development of nitrate tolerance.

Acknowledgments

Dr Kemp-Harper is a CJ Martin Research Fellow and Dr Favaloro a Peter Doherty Fellow of the NHMRC (Australia). Their support is gratefully acknowledged.

References


32. Mistry DJ, Garland CJ. Nitric oxide (NO)-induced activation of large conductance Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels (BK\textsubscript{Ca}) in smooth muscle cells isolated from the rat mesenteric artery. *Br J Pharmacol.* 1998;124:1131–1140.


NO- Activates Soluble Guanylate Cyclase and Kv Channels to Vasodilate Resistance Arteries
Jennifer C. Irvine, Joanne L. Favaloro and Barbara K. Kemp-Harper

Hypertension. 2003;41:1301-1307; originally published online May 12, 2003;
doi: 10.1161/01.HYP.0000072010.54901.DE
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/41/6/1301

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2003/05/22/41.6.1301.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/