Use of a Nitronyl Nitroxide to Discriminate the Contribution of Nitric Oxide Radical in Endothelium-Dependent Relaxation of Control and Diabetic Blood Vessels

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ABSTRACT

Nitronyl nitroxides react with nitric oxide radical (NO) to form imino nitroxides. We used a nitronyl nitroxide, [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3 oxide] (CPTIO) to evaluate the contribution of NO to basal tone and acetylcholine-induced endothelium-dependent relaxation in control vs. diabetic rat aortic rings. In rings precontracted with phenylephrine, CPTIO produced an additional increment in tension that was greater in control vs. diabetic rings. Tension after CPTIO was similar to that observed in rings pretreated with the NO synthase inhibitor, L-nitroarginine or in rings without endothelium. This increment was insensitive to indomethacin, cysteine, tetraethylammonium or catalase, but was sensitive to inhibition by the soluble guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one. L-Nitroarginine blocked relaxation to ACH by 100 and 90% in control and diabetic rings, respectively. In contrast, CPTIO produced a concentration-dependent inhibition of ACH-induced relaxation that was greater in control rings. The residual CPTIO-resistant component of relaxation was equivalent to 26 and 43% of initial precontraction in control vs. diabetic rings, respectively, and was not altered by indomethacin, catalase, cysteine or tetraethylammonium but was significantly inhibited by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one. These data suggest the release of additional unknown factor(s) that cannot be discerned using NO synthase inhibitors only. This CPTIO-resistant dilator is likely not a cyclooxygenase product or a hyperpolarizing factor but a factor that acts, in part, by activation of guanylate cyclase. This substance is possibly •NO that is not available for reaction with CPTIO either by its diffusibility and sequestration or molecular rearrangement to a redox active form (i.e., not free •NO) or is a completely different vasodilator. The use of a more lipid soluble nitronyl nitroxide derivative suggests a portion of the CPTIO-resistant relaxation in diabetic (but not control) rings could be explained by •NO sequestered in the lipid phase.

Furchgott and Zawadski (1980) were the first to report the phenomenon of endothelium-dependent relaxation of blood vessels. It has been suggested that this action results from the release of an EDRF that was likely to be NO or a closely related entity (Palmer et al., 1988). Since these initial reports, it has become apparent that the endothelium-dependent relaxation of certain blood vessels to certain stimuli is not entirely mediated by EDRF/NO but may also include other relaxation factors such as EDHF or prostanoids (Cohen and Vanhoutte, 1995).

Arginine analogs that are competitive antagonists to NOS have been used to assess the contribution of NO to relaxation induced by a variety of endothelium-dependent agonists. These compounds do not antagonize NO directly but only block NO synthesis via NOS. A different approach to assess the role of NO is by using pharmacological probes that directly react with or scavenge NO. Indeed, we have previously reported that the iron-thiol containing NO scavenger, MGDFe, significantly attenuated ACH-stimulated relaxation in both control and diabetic rat aortic rings; however, a significant component of relaxation was resistant to MGDFe antagonism (Pieper and Lai, 1997). These observations were interesting because ACH-mediated relaxation in both experimental groups is virtually abolished by L-NA and relaxation to ACH is not modified by indomethacin (Pieper et al., 1997). Taken together, these studies raise some question whether endothelium-dependent relation in this model is, in fact, entirely mediated by NO radical (•NO) per se.

To further understand this discrepancy, we used an agent belonging to a uniquely different class of NO antagonist known as nitronyl nitroxides. One of these nitronyl nitrox-
sustained phase of relaxation to ACH since the factors or molecular inter- 
ventions altered either the initial rapid phase vs. maximum NOS-dependent, endothelium-dependent relaxation to ACH lumen with a forceps. Rings, the endothelium was intentionally removed by rubbing the 
tissue, 1995). Diabetes was verified at 1 wk using an Exac Tech glu-
counter the specific role of species responsible for hyperpolarization and relaxation has been 
suggested to be different at each phase (Vanheel et al., 1994; Rubanyi et al., 1985).

To evaluate the CPTIO-resistant component of ACH-mediated relaxation, we also performed several experiments using specific drug interventions. These included 10 μM indomethacin (to inhibit cyclooxygenase); 100 μM L-NA (to inhibit NOS); 1 mM TEA (to inhibit K\(^+\) channels); 1 mM L-cysteine (to scavenge nitroxyl anion and/or peroxynitrite anion); 1000 U/ml catalase (to decompose H\(_2\)O\(_2\)) and 3 μM of ODQ to inhibit soluble guanylate cyclase (Garthwaite et al., 1995, Moro et al., 1996).

Data are presented as the mean ± S.E.M. where n equal the number of individual animals. Data were analyzed by analysis of variance or repeated analysis of variance followed by Fishers PLSD test for multiple mean comparisons, where appropriate; or by unpaired t test and paired t test for comparison of two group means, where appropriate. A P < .05 was considered to designate statistical significance.

**Materials and Methods**

Male Sprague-Dawley rats were made diabetic by an i.v. injection of 55 mg/kg streptozotocin as previously described (Pieper and Pel- 
tier, 1995). Diabetes was verified at 1 wk using an Exac Tech glu-
cometer and test strips. Diabetic and age-matched control rats were 
mounted between parallel wires in isolated tissue baths at 37°C. The bath medium contained Krebs bicarbonate buffer which was oxygenated at 95%/5% O\(_2\)/CO\(_2\), to maintain pH at 7.4. The buffer contained (in mM): NaCl 118, KCl 4.7, CaCl\(_2\) 2.5, MgSO\(_4\) 1.2, 
HCO\(_3\)-24 and glucose 10.5. Rings were equilibrated under optimal 
tensions of 2.0 g for both control and diabetic rings before performing 
measurements of contractile reactivity to PE. Isometric tension were 
recorded on a Gould (Valley View, OH) TA6000 recorder using Rad-
ti (Monrovia, CA) model 159901 force-displacement transducers.

Each ring was contracted with cumulative concentrations of PE. After washing and equilibrium, rings were contracted with a sub-
ificant role of NO vs. EDHF that contributes to total ACH-induced relaxation. This 
was no increase in tension following addition of CPTIO in rings under resting tension (i.e., without PE, data not shown). The incremental change in CPTIO-induced tension development was greater in control compared to diabetic rings (example for 300 μM CPTIO shown in fig. 1 and fig. 7, A and C). Similar differences between control and diabetic rings were seen using 300 μM PTIO (not shown). The CPTIO-
duced increment in tension was not modified by prior incu-
tration with either indomethacin, TEA, cysteine or catalase (fig. 1). Although the increment was slightly reduced by cys-
teine in diabetic rings compared to the entire group without 
cysteine (P < .05), this effect was not seen if analyzed com-
de the smaller subset of pair-matched rings (P = 2.229, not significant). In contrast, previous incubation with ODQ markedly inhibited CPTIO-induced tension development by 83 and 86% in control vs. diabetic rings, respectively. Simi-
larly, the addition of the combination of ODQ with either indomethacin, TEA and catalase produced no further inhibition of CPTIO-induced tension than by ODQ alone.

| TABLE 1 | Contractile effects of phenylephrine in control vs. diabetic aortic rings |
|---|---|---|---|
| | Maximum (g) | Maximum (g/mm\(^2\)) | pD\(_2\) |
| **With endothelium** | | | |
| Control (n = 30) | 1.41 ± 0.09 | 1.50 ± 0.09 | 6.33 ± 0.03 |
| Diabetic (n = 33) | 1.08 ± 0.07 | 1.48 ± 0.07 | 6.14 ± 0.04 |
| P < .005 N.S. | P < .001 |
| **Without endothelium** | | | |
| Control (n = 12) | 2.82 ± 0.10 | 3.19 ± 0.10 | 7.04 ± 0.10 |
| Diabetic (n = 12) | 1.83 ± 0.16 | 2.67 ± 0.22 | 6.78 ± 0.10 |
| P < .001 N.S. | N.S. N.S. |

N.S., Not significant, P > .05.
At the highest concentration of CPTIO tested (i.e., 300 μM), the tension development was similar to that seen in rings without endothelium but without CPTIO or in rings pretreated with L-NA (fig. 2). Addition of CPTIO to rings without endothelium or in rings with endothelium but pretreated with L-NA (control: n = 12; diabetic: n = 11) produced no significant increase in tension development (not shown).

Vascular relaxation analysis. For all rings, the 1 μM PE concentration used for the relaxation studies produced 66 ± 2 and 58 ± 2% maximum tension for control vs. diabetic rings, respectively. ACH fully relaxed both control and diabetic rings (examples shown in fig. 7, b and d) and this relaxation was significantly inhibited by L-NA, ODQ or by removal of the endothelium in both groups (fig. 3). Although blockade of ACH -mediated relaxation by L-NA and ODQ was complete in control rings, a significant residual component of ACH-stimulated relaxation was present in diabetic rings equivalent to 15 ± 5 and 27 ± 6% in L-NA-treated and ODQ-treated rings, respectively. In contrast, ODQ caused complete and equivalent inhibition of nitroglycerin-induced relaxation in both control and diabetic rings (not shown).

Effects of CPTIO on ACH-induced relaxation. Addition of 30 to 300 μM CPTIO to control rings caused a concentration-dependent inhibition in the ACH-induced relaxation (fig. 4). Addition of 500 μM CPTIO caused no further inhibition (not shown). CPTIO also caused a concentration-dependent inhibition of relaxation in diabetic rings (not shown). We used 300 μM CPTIO to further evaluate the role of NO in ACH-mediated relaxation of control and diabetic rings. The % residual ACH-induced relaxation that was resistant to CPTIO was greater in diabetic rings than in control rings (fig. 5). Addition of either indomethacin (not shown), catalase or TEA or L-cysteine did not alter the CPTIO-resistant component of ACH-induced relaxation in both control and diabetic rings (fig. 6). In contrast, the CPTIO-resistant component of ACH-mediated relaxation was inhibited completely by preincubation with ODQ in control rings but left a small residual portion of relaxation in diabetic rings (fig. 6). Although pre-treatment with CPTIO alone before addition of increasing concentrations of ACH partially inhibited the relaxation in control and diabetic rings, the addition of CPTIO after addition of the highest concentration of ACH completely restored tension in both control and diabetic rings to levels greater than that observed prior to the addition of ACH (fig. 7).

To gather additional information regarding the nature of the CPTIO-resistant portion of ACH-mediated relaxation, we challenged both control and diabetic rings with a single concentration of ACH (i.e., 30 μM) rather than cumulative ACH concentrations. Despite total relaxation of both control and diabetic rings with this single ACH concentration (not
pretreatment with 300 μM CPTIO inhibited relaxation to ACH in control (to 17 ± 1% residual relaxation, n = 7) and diabetic rings (to 35 ± 4% residual relaxation, n = 12) (fig. 8). Addition of 30 μM ACH produced an initial rapid phase of relaxation in rings pretreated with CPTIO. The peak CPTIO-resistant portion of relaxation to 30 μM ACH produced an initial rapid phase of relaxation in rings pretreated with CPTIO. The peak CPTIO-resistant portion of relaxation to 30 μM ACH was unaltered in both control and diabetic rings by prior treatment with either indomethacin or TEA or catalase (fig. 8). In contrast, the CPTIO-resistant component of relaxation to ACH was essentially abolished in ODQ-treated control (i.e., 3.5 ± 1.5% residual relaxation) and significantly, but only partially, reduced in ODQ-treated diabetic rings (i.e., 11 ± 2% residual relaxation). Superimposition of either indomethacin, TEA or catalase in combination with ODQ did not further modify the responses seen in CPTIO-treated, ODQ-treated rings (not shown).

To evaluate the effects of another nitronyl nitroxide, we repeated the studies using a challenge with increasing concentrations of ACH. Substitution with 300 μM PTIO caused inhibition of ACH-mediated relaxation in control and diabetic rings (fig. 9). The combination of both CPTIO and PTIO (300 μM each) did not cause any further inhibition of ACH-mediated relaxation of control rings than that achieved by CPTIO or PTIO alone (fig. 9, upper panel). In contrast in diabetic rings, PTIO alone or PTIO in combination with CPTIO caused a greater inhibition of relaxation to ACH than did CPTIO alone (fig. 9, lower panel).
with CPTIO alone, the residual relaxation to ACH that was insensitive to PTIO or CPTIO + PTIO was not different between control vs. diabetic rings. Nevertheless, a significant portion in ACH-mediated relaxation (approximately 20%) still remained in both control and diabetic rings despite the addition of PTIO.

**Discussion**

In our study, we have characterized the contribution of NO to basal tone and ACH-stimulated endothelium-dependent relaxation of control and diabetic rat aortic rings. Our studies suggest that the nitronyl nitroxide, CPTIO, antagonized all of the NO released under basal conditions in both control and diabetic rings. In addition, CPTIO inhibited a significant portion of ACH-stimulated relaxation in both control and diabetic rings; however, a residual component of relaxation remained which was larger in diabetic rings. This effect occurred despite the observation that relaxation was virtually eliminated in both groups using the NOS inhibitor, L-NA. Although our study is the first known application of the nitronyl nitroxide class of NO antagonists to provide valuable insight into the nature of endothelium-dependent relaxation in diseased blood vessels, it is clear that these agents give important information about NO-like activity that would not be achieved using NOS inhibitors alone.

**Fig. 6.** Failure of incubation with 1 mM TEA (control: \( n = 5 \); diabetic: \( n = 6 \)), 1 mM L-cysteine (control: \( n = 4 \); diabetic: \( n = 5 \)), or 1,000 U/ml catalase (control: \( n = 5 \); diabetic: \( n = 6 \)) but not 3 \( \mu \)M ODQ (control: \( n = 3 \); diabetic: \( n = 6 \)), to alter the ACH-induced relaxation component resistant to antagonism by 300 \( \mu \)M CPTIO in control and diabetic rings. TEA. *\( P < .05 \) vs. pair-matched, untreated rings with CPTIO alone.
Basal NO tone. Our studies suggest that NO per se is the likely product released by both control and diabetic rings under control unstimulated conditions and that the nitronyl nitroxide, CPTIO, is effective in counteracting all of the NO activity released under these conditions. This conclusion is based on several observations including: 1) the CPTIO-sensitive incremental increase of tension development in PE-contracted rings was insensitive to indomethacin, cysteine, TEA and catalase but was inhibited by ODQ; 2) the CPTIO-sensitive component of tension development was equivalent to that achieved by removal of the endothelium or by pretreatment with L-NA and 3) the addition of CPTIO to rings without endothelium or L-NA-treated rings with endothelium did not produce any significant change in tension.

This effect on PE-induced contractile tone is not unique to the nitronyl nitroxide CPTIO since we have observed that the iron-thiol-containing scavenger of NO (e.g., MGDFe) also completely scavenged basal NO activity (Pieper and Lai, 1996). The observation that the incremental increase in tension in response to both CPTIO and MGDFe was greater in control vs. diabetic rings and that treatment with L-NA or removal of endothelium produces a greater increase in tension in control vs. diabetic rings without CPTIO (this study) or MGDFe (Pieper and Lai, 1997) suggests reduced basal NO activity in diabetic blood vessels.

Agonist-stimulated endothelium-dependent relaxation in diabetes. Endothelium-dependent relaxation to ACH and other agonists is impaired in a variety of conduit and resistance blood vessels taken from experimental diabetic animals. Because endothelium-dependent relaxation is also impaired in both type I (Johnstone et al., 1993) and type II (McVeigh et al., 1992) diabetes mellitus in humans, information derived from experimental diabetic models may provide insight to the defect in human diabetes. Several studies using a variety of vessels from different species show that indomethacin does not shift the relaxation responses to ACH (Oyama et al., 1986; Hattori et al., 1991; Cameron and Cotter, 1992; Chang and Stevens, 1992; Pieper et al., 1992, 1997; Mayhan, 1992; Dai et al., 1993; Taylor et al., 1994, Diederich et al., 1994; Kamata and Kobayashi, 1996). These collective observations suggest that prostanoid factors cannot account for this dysfunction and that non-prostanoid factors such as deficits in NO per se can readily account for endothelial dysfunction in diabetes.

Role of NO in diminished endothelium-dependent relaxation in diabetes. In support of deficits in NO activ-
Potential role of an aberrant NOS reaction/reaction products. In the presence of an arginine deficiency or limited cofactor, purified NOS enzyme can reduce molecular oxygen to $\text{H}_2\text{O}_2$ or superoxide anion radical accompanied by diminished production of $\text{NO}$ (Heinzel et al., 1992; Pou et al., 1992). This may have some implications in diabetic blood vessels. Indeed, the concentration of tetrahydrobiopterin has been shown to be diminished in brain tissue of diabetic animals (Hamon et al., 1989) although supplementation with a tetrahydrobiopterin derivative in vitro restores endothelium-dependent relaxation to ACH in diabetic rat aorta (Pieper, 1997). In addition, we have shown decreases in plasma arginine concentration (Pieper and Peltier, 1995) and in arginine content in vascular tissue of diabetic rats (Pieper and Dondlinger, 1996). Furthermore, supplementation with L-arginine in vitro or in vivo restores endothelium-dependent relaxation to ACH in diabetic rat aortic rings and improves cGMP generation (Pieper and Peltier, 1995; Pieper and Dondlinger, 1996; Pieper et al., 1996a). We have also shown that superoxide dismutase plus catalase restores ACH-induced relaxation to normal in diabetic aortic rings (Pieper et al., 1997) which would be consistent with increased basal production of superoxide anion radicals, $\cdot \text{O}_2^-$ (Chang et al., 1993; Pieper, 1995) and $\text{H}_2\text{O}_2$ (Pieper, 1995) by diabetic aorta. Thus, it is theoretically possible that an aberrant NOS may be a source of increased reactive oxygen production from diabetic endothelium.

Comparison of the effects of nitronyl nitroxide vs. NOS inhibitors. The CPTIO-resistant component of relaxation to ACH that was greater in diabetic vs. control rings is not unique to CPTIO because a similar observation was shown using MGDFe (Pieper and Lai, 1997), an agent that acts to interact with NO in a manner quite different than that achieved by CPTIO. Our use of a nitronyl nitroxide compared with L-NA appears be a useful candidate probe to discriminate the effects of $\text{NO}$ vs. other products of the NOS reaction on endothelium-dependent relaxation in control vs. diabetic blood vessels. Reliance alone on the efficacy of L-NA to inhibit relaxation to ACH in both control and diabetic rings does not prove that relaxations are, in fact, entirely mediated via $\cdot \text{NO}$. Rather, it simply suggests that relaxation arises from a NOS pathway. Indeed, L-NA might also eliminate the reactive oxygen by-products of the NOS reaction as well. We suggest, therefore, that a portion of the L-NA-sensitive, ACH-mediated relaxation that was insensitive to CPTIO and larger in diabetic rings might result from increased generation of products arising from an aberrant NOS reaction in diseased blood vessels.

One possibility to explain the larger CPTIO-insensitive component of relaxation to ACH in diabetic blood vessels is that $\text{H}_2\text{O}_2$ as well as the product of $\cdot \text{O}_2^-$ and $\cdot \text{NO}$, known as ONOO$^-$ are also vasodilators (Wei et al., 1996; Liu et al., 1994) that can activate guanylate cyclase (Tarpey et al., 1995). This explanation is attractive based on observation of enhanced rates of $\cdot \text{O}_2^-$ and $\text{H}_2\text{O}_2$ production in diabetic rat aorta (Pieper, 1995). At present, we cannot exclude the possibility that production of ONOO$^-$ by diabetic rings could impact CPTIO activity because nitronyl nitroxides are sus-

![Graph of CONTROL RINGS and DIABETIC RINGS](image)

Fig. 9. Failure of substitution with 300 $\mu$M PTIO or addition of PTIO plus 300 $\mu$M CPTIO to alter the CPTIO-resistant portion of acetylcholine-induced relaxation in control rings ($n = 5$ or $8$) but not diabetic rings ($n = 6$–7 each). *$P < .05$ vs. pair-matched rings treated with CPTIO alone.
ceptible to reduction by reactive oxygen (Akaike and Maeda, 1996). In this case, there would be less active CPTIO available to react with and neutralize ·NO. We believe that this explanation would be insufficient because the addition of CPTIO at the end of ACH completely restored tension in both control and diabetic rings. This could be explained by the rapid decay of an ·NO-independent factor released initially upon ACH stimulation.

Our additional studies do suggest that a major portion of the CPTIO-resistant portion of ACH-induced relaxation in both control and diabetic rings is likely mediated by a substance that activates soluble guanylate cyclase based on the studies using ODQ. Unlike methylene blue, ODQ is a potent, highly selective inhibitor of soluble guanylate cyclase that also does not alter NOS activity (Brunner et al., 1996). The actions of ODQ to block the CPTIO-resistant portion of relaxation to ACH would also be consistent with the actions of both H_2O_2 or ONOO⁻ as candidate molecules especially in diabetic blood vessels. Thus, taken together with the studies using L-NA, our data suggest that a greater proportion of ACH-mediated relaxation in diabetic blood vessels occurs via a substance or factor that: 1) arises from the NOS reaction, 2) is insensitive to antagonism by CPTIO, 3) is an activator of guanylate cyclase, and 4) may not be ·NO.

Potential role of EDHF. We considered the possibility that the CPTIO-resistant component of relaxation to ACH is related to release of an EDHF that may act via cGMP-dependent or cGMP-independent pathways (Cohen and Vanhoutte, 1995). Previous studies discounted a role of alterations in EDHF to account for impaired endothelium-dependent relaxation in diabetic rat aorta (Endo et al., 1995). In our study, our endothelium-dependent relaxation protocols using CPTIO in the presence of indomethacin, TEA or catalase suggest that any putative alteration in prostanooid production, activation of calcium-activated K⁺ channel-sensitive hyperpolarizing factor or H_2O_2 generation in diabetic rings is unlikely to account for this larger CPTIO-resistant component of relaxation to ACH in diabetic compared to control rings.

Potential role of nitrosothiols. We also considered the possibility that a portion of the relaxation produced by ACH arises from release of a thiol-bound derivative such as S-nitrosocysteine (Myers et al., 1990; Rubanyi et al., 1991) because CPTIO is unable to interact chemically with nitrosothiols (Akaike and Maeda, 1996). Reaction of ·NO with cysteine produces S-nitrosocysteine that increases the potency of authentic EDRF and increases its half-life. In this regard, l-cysteine has been shown to potentiate ·NO-dependent relaxation but inhibit relaxation mediated by NO (Pino and Fiebel, 1994) and might also interact with ONOO⁻ (Pfeiffer et al., 1997). Because l-cysteine did not change the CPTIO-resistant portion of relaxation to ACH in either control or diabetic rings, we conclude that the CPTIO-resistant portion of relaxation to ACH in diabetic rings is likely not mediated by ONOO⁻.

We also considered the possibility that the CPTIO-resistant portion of relaxation to ACH is mediated by the product of reaction of endothelial-derived ·NO with CPTIO. In this regard, the reaction of CPTIO with ·NO is a radical-radical reaction producing the products, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1 oxyl (known as CPTI) and NO₂. CPTI caused vasodilation in canine coronary arteries (Tsunoda et al., 1994) that was inhibited by methylene blue suggesting activation of soluble guanylate cyclase, this would be consistent with our results showing that the CPTIO-resistant component is sensitive to ODQ. This explanation would be inadequate to explain the CPTIO-resistant relaxation to ACH particularly in diabetic rings for two reasons. First, the lipophilic nitroso nitroxide, PTIO, used alone or in combination with CPTIO (see below) caused further inhibition. Second, addition of CPTIO after addition of ACH completely abolished ACH-mediated relaxation in both groups and restored tension to above baseline.

Role of sequestration of ·NO in the lipid phase. Previous cell-free studies using nitroxide compounds (rather than nitroso nitroxides) suggest that ·NO might partition in lipid environments (Singh et al., 1994). In fact, at least two reports suggest that ·NO might be sequestered in large quantities in lipid bilayers (Lancaster, 1996; Deniciola et al., 1996). Thus, we considered the possibility that some of the ·NO escapes reaction with CPTIO (as with our previous studies using MGDFe) due to the fact that both are predominately water-soluble agents and, therefore, might be unable to effectively counteract the high diffusibility of ·NO under agonist-stimulated conditions.

To evaluate this contingency, we performed additional experiments using a structurally related ·NO antagonist, PTIO, which has a 100-fold increase in lipophilicity. PTIO used either alone or in combination with CPTIO also failed to alter relaxation to ACH in control rings suggesting that lipid sequestration might not account for the portion resistant to relaxation in normal blood vessels. Interestingly, the addition of PTIO partially inhibited the relaxation to ACH only in diabetic rings and in an amount greater than that achieved by CPTIO alone. The results in control rings show that there was no difference in the degree of inhibition of relaxation to ACH by PTIO and CPTIO suggesting that this could not be explained simply on an intrinsic difference in potency between the two nitroso nitroxides.

Alternatively, potential alterations in the lipid composition in diabetic membranes might sequester more PTIO than in control membranes. This is an attractive explanation for other reasons because this lipid environment could serve as a reservoir for ·NO increasing the probability of inactivation by interaction with fatty acids, especially peroxyl radicals that are known to react with ·NO (Padmaja and Huie, 1993). This would reduce the effective ·NO activity and be consistent with the observation of decreased endothelium-dependent relaxation in diabetic blood vessels. Nevertheless, the potential increased sequestration cannot account for all of the (CPTIO + PTIO)-resistant portion of relaxation to ACH because a significant portion equivalent to 30% remained in both control and diabetic rings.

In summary, use of nitroso nitroxides rather than reliance solely on NOS inhibition has allowed a greater understanding of the role of ·NO in endothelium-dependent relaxation in control and diabetic blood vessels. Our studies suggest a large portion of relaxation to ACH in control blood vessels is mediated by ·NO but an additional portion that is larger in diabetic blood vessels may be another species derived either directly or indirectly from the NOS reaction. Furthermore, our studies suggest that the lipid fraction of diabetic blood vessels may serve as a larger sink for sequestration of ·NO that may potentially limit its biological action on diabetic vascular smooth muscle.
References


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