Biological Activity of Designed Photolabile Metal Nitrosyls: Light-Dependent Activation of Soluble Guanylate Cyclase and Vasorelaxant Properties in Rat Aorta

Melanie Madhani,[†] Apurba K. Patra,[‡] Thomas W. Miller,[§] Aura A. Eroy-Reveles,[‡] Adrian J. Hobbs,[†] Jon M. Fukuto,*[,||] and Pradip K. Mascharak*[,‡]

Wolfson Institute for Biomedical Research, University College London, Cruciform Building, Gower Street, London WC1E 6AE, United Kingdom, Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90095, and Department of Pharmacology, University of California, Los Angeles School of Medicine, Center for the Health Sciences, Los Angeles, California 90095

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The biological and pharmacological utility of nitric oxide (NO) has led to the development of many classes of NO-donor compounds as both research tools and therapeutic agents. Many donors currently in use rely on thermal decomposition or bioactivation for the release of NO. We have developed several photolabile metal-nitrosyl donors that release NO when exposed to either visible or UV light. Herein, we show that these donors are capable of activating the primary "NO receptor", soluble guanylate cyclase (sGC), in a light-dependent fashion leading to increases in cGMP. Moreover, we demonstrate that these donors are capable of eliciting light-dependent increases of cGMP in smooth muscle cells and vasorelaxation of rat aortic smooth muscle tissue, all effects that are attributed to activation of sGC. The potential utility of these compounds as drugs and/or research tools is discussed.

Introduction

Nitric oxide (NO) regulates an array of physiological processes including the maintenance of vascular tone, the control of platelet and leukocyte reactivity, mitogenesis, penile erection, gastrointestinal motility, and central neurotransmission.[1] The biological activity of NO in these systems, generated at relatively low concentrations (possibly ranging from 2 to 300 nM)[2] by the constitutive isoforms of NO synthase (NOS), is almost exclusively the result of a direct activation of the enzyme soluble guanylate cyclase (sGC). This heterodimeric hemoprotein catalyzes the formation of the second messenger, cyclic guanosine-3′,5′-monophosphate (cGMP), which governs many aspects of cellular function via interaction with cGMP-dependent proteins, kinases, cyclic nucleotide gated ion channels, or cyclic nucleotide phosphodiesterases.[3,4] In contrast, under pathological conditions, "high-output" NO production (low micromolar)[5] by the inducible isoform of NOS (iNOS) results in considerable cGMP-independent activity such as inhibition of respiratory complexes and aconitase, *S*-nitrosation, DNA modification, and cell apoptosis.[6,7] This type of activity is crucial to the cytotoxic actions of NO that constitutes an important aspect of host defense.

As a consequence of its multifaceted role in mammalian physiology, inappropriate NO production/activity has been hypothesized to initiate, or contribute to, the development of a number of disorders including hypertension, atherosclerosis, stroke, migraine, and erectile dysfunction.[6] This has led to the development of therapeutics that augment or inhibit endogenous NO production. While considerable developmental effort has focused on the identification of NOS inhibitors, their potential as medicines has yet to be exploited. In marked contrast, NO-donor drugs (for example, nitrovasodilators) have been used in clinical practice for over a century for the treatment of cardiovascular disorders such as angina and heart failure.[8] However, this class of therapeutic, exemplified by the organic nitrates glyceryl trinitrate (GTN) and isosorbide dinitrate, is administered predominantly in a systemic fashion. Because NO is a lipophilic molecule, virtually all cells in the body are exposed to an increased NO concentration following such administration, and this is manifested acutely as side effects such as headaches and postural hypotension (as a result of central and peripheral vasodilatation). Other potentially detrimental effects of chronic NO administration such as inhibition of oxidative phosphorylation and DNA modification also remain as problems. Moreover, nitrate therapy is plagued by the development of tolerance following prolonged administration.[8,9] Thus, there is a clear need for the development of novel NO-donor drugs that might be targeted to particular organs/tissues and release NO in a controlled fashion to bring about selective therapy. For example, tissue selective and timely generation of NO might be important in the utilization of NO donors in photodynamic and cancer radiation therapy. Moreover, topical release of NO might be useful (a) in the treatment of conditions associated with peripheral vasculopathies such as Raynaud's disease and diabetes mellitus, contact dermatitis, and (b) to accelerate wound healing and perhaps to rectify pigment variations (vide infra).

There has been significant interest in the development of photolabile NO donors because this can be a means of controlled and selective NO release.[10] In particular, the Ford lab[11−18] along with others[19−23] have developed and examined many compounds whose release of NO is dependent on photoactivation. Herein we report several new photolabile, metal nitrosyl NO donors that we have synthesized and characterized both chemically and biologically. These donors rapidly release NO when exposed to specific wavelengths of light of medium-to-low intensity (50 W to 5 mW).[24−29] These nitrosyls have the composition of $[(L)M(NO)]^{n+}$, where M = Fe, Ru, or Mn and L = pentadentate

* To whom correspondence should be addressed. Tel.: (831) 459 4251 (P.K.M.); (310) 206 7151 (J.M.F.). Fax: (831) 459 2935 (P.K.M.); (310) 825 6267 (J.M.F.). E-mail: mascharak@chemistry.ucsc.edu (P.K.M.); jfukuto@mednet.ucla.edu (J.M.F.).

[†] University College London.

[‡] University of California, Santa Cruz.

[§] University of California, Los Angeles, Department of Chemistry and Biochemistry.

[||] University of California, Los Angeles, Department of Pharmacology.

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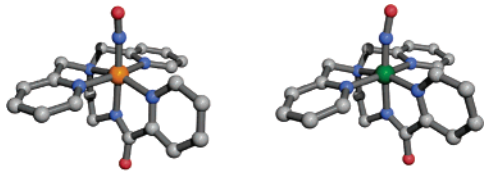
polypyridine ligands containing one carboxamide group. Two NO donors of this family, namely, $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**; structures shown) have been studied in detail in terms of their capacity to release NO and activate biological targets. The ruthenium nitrosyl complex **1** is soluble in water and stable under ordinary light conditions but releases NO upon exposure to low-intensity UV light (5−7 mW). In aqueous solution, the photoproduct has been identified as $[Ru^{III}(PaPy_3)(H_2O)]^{2+}$ (eq 1).[29] This reaction has been employed to efficiently transfer NO to biological targets like reduced cytochrome c oxidase and reduced myoglobin under the strict control of UV illumination.[29] The manganese nitrosyl **2** is also soluble in water and releases NO upon illumination by visible light (eq 2).[27] Complex **2** has been shown to inhibit papain via phototransfer of NO to the active site cysteine when a mixture of papain and **2** is exposed to visible light.[28]

$$[Ru(PaPy_3)(NO)]^{2+} + h\nu \rightarrow [Ru^{III}(PaPy_3)(H_2O)]^{2+} + NO \quad (1)$$

$$[Mn(PaPy_3)(NO)]^{+} + h\nu \rightarrow [Mn^{II}(PaPy_3)(H_2O)]^{+} + NO \quad (2)$$

As further exploration of the biochemical and pharmacological characterization of $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**), we herein report their light-dependent (bio)activity with respect to activation of purified sGC, stimulation of cGMP production in vascular smooth muscle cells in culture and vasorelaxation in rat aorta in vitro.



$[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**)
Orange = Ru, gray = C,
blue = N, red = O

$[Mn(PaPy_3)(NO)]ClO_4$ (**2**)
Green = Mn, gray = C,
blue = N, red = O

Materials and Methods

Synthesis. $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**) were synthesized by following previously published procedures.[27,29]

Chemicals/Solutions. All biochemical reagents were purchased from Sigma except spermine NONOate (SPER-NO; Calbiochem). The cGMP EIA immunoassay kit (RPN226) and NAP-10 columns were purchased from AP Biosciences.

Expression of sGC. Both the α_1 and β_1 subunits of human sGC were expressed in a baculovirus expression system. The human sGCα_1 cDNA (kind gift of Bayer AG, Germany) was cloned into a baculovirus transfer vector (pBlueBac 4.5, Invitrogen) using BamHI/HindIII restriction sites and an N-terminal HIS_6 tag incorporated by PCR (using primers that amplified the sequence containing the transcription start site but additionally possessing a HIS_6 repeat). The β_1 subunit cDNA (kind gift of Bayer AG, Germany) was cloned into pBlueBac 4.5 using EcoRI. Recombinant virus was then generated from both constructs using a commercially available kit (Bac-N-Blue, Invitrogen). The recombinant viruses were plaque-purified and high-titer ($>10^8$ pfu/mL) stocks were obtained from *Spodoptera frugiperda* (*Sf*21) cells using several rounds of infection and stored at 4 °C until use. For expression of recombinant protein, *Sf*21 cells were grown as spinner cultures and then plated in 500 cm^2 dishes in TC100 medium containing 10% FBS (New Zealand) and a penicillin/streptomycin mix. Cells were left to adhere for 24 h before being infected at a 1:1 ratio of sGCα/sGCβ with a final MOI of five in the presence of 2.5 μg/mL hemin. Cells were harvested after 72 h, washed three times in PBS, and resuspended in lysis buffer (50 mM triethanolamine-HCl, pH 7.4, 300 mM NaCl, 10 mM imidazole, 1% Igepal, and a peptidase inhibitor cocktail consisting of 10 μg/mL aprotonin, pepstatin, leupeptin, and benzamidine). Cells were kept on ice for 30 min to allow efficient lysis and a cytosolic fraction obtained by centrifugation at 100 000 × g for 60 min at 4 °C.

sGC Purification. To purify the recombinant dimeric protein, the cytosolic fraction was mixed with NiNTA resin (Qiagen; 5 mL lysate to 1 mL 50% resin) and batch-bound at 4 °C for 1 h. The resin/lysates mix was then loaded onto a 10 mL column and washed with four bed volumes of wash buffer (50 mM triethanolamine-HCl, pH 7.4, 300 mM NaCl, 10 mM imidazole). The human sGC protein was stripped from the column using elution buffer (50 mM triethanolamine-HCl, pH 7.4, 30 mM NaCl, 1 mM dithiothreitol, and 150 mM imidazole), and 1 mL fractions were collected. Fractions containing sGC (identified by immunoblotting using antibodies directed against the human α_1 and β_1 subunits, kind gift of Pfizer, U.K.) were pooled and applied to a pre-equilibrated (50 mM triethanolamine-HCl, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol) Mono-Q anion exchange column (10 mL; Amersham biosciences) at 1 mL/min. The column was then washed with three bed volumes of wash buffer (50 mM triethanolamine-HCl, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol), and the sGC protein was eluted by a salt gradient (0.1−1 M NaCl in 3 bed volumes) and 1 mL fractions were collected. Fractions containing sGC (identified by immunoblotting) were pooled and used for experimentation.

Light-Induced Release of NO from the Nitrosyl Complexes. Photochemical release of NO from the manganese nitrosyl **2** was accomplished by using either a tungsten or a halogen lamp. Release of NO from the ruthenium nitrosyl **1** was accomplished with the use of a hand-held UV lamp (Ultra Violet Products, model UVSL-25) operating at 254 nm (4w). Typically, solutions were illuminated directly prior to the measurements (sGC activity, cGMP levels, vasorelaxation).

Measurement of sGC Activity. Enzyme activity was determined by measuring the conversion of GTP to cGMP in the absence and presence of the NO donors. Briefly, isolated enzyme (~0.2 μg protein) was incubated in reaction buffer (50 mM triethanolamine-HCl, pH 7.4) containing IBMX (1 mM), phosphocreatine (5 mM), creatine kinase (5U/mL), $MgCl_2$ (4 mM), BSA (1 mg/mL), dithiothreitol (1 mM), and GTP (2 mM) at 37 °C for 10 min. The reaction was terminated by the addition of 50 mM ice-cold sodium acetate (pH 4.0) and then heating at 95 °C for 3 min. Cyclic GMP accumulation was measured using a commercially available ELISA kit (Amersham Biosciences), as per the manufacturer instructions.

Tissue Bath Experiments. Male rats (200−250 g, Sprague−Dawley) were stunned and killed by cervical dislocation. The thoracic aortas were carefully removed, cleaned of connective tissue, and cut into 3−4 ring segments of approximately 4 mm in length. Aortic rings were mounted in 10 mL organ baths containing Krebs-bicarbonate buffer (composition (mM): Na^+, 143; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 128; HCO_3^-, 25; HPO_4^{2-}, 1.2; SO_4^{2-}, 1.2; and D-glucose, 11), maintained at 37 °C and gassed with 95% O_2/5% CO_2. Tension was initially set at 1 g and reset at intervals, following an equilibration period of 1 h, during which time fresh Krebs-bicarbonate buffer was replaced every 15−20 min. After equilibration, the rings were primed with KCl (4.8×10^{-2} M) before a supramaximal concentration of phenylephrine (PE; 10^{-6} M) was added. Once this response had stabilized, acetylcholine (ACh; 10^{-6} M) was added to the bath to assess the integrity of endothelium of WT vessels. If the contractions to PE were not maintained or relaxations greater than 50% of the PE-induced tone to ACh were not observed, the tissues were discarded.

Tissues were then washed for 30 min (by addition of fresh Krebs-bicarbonate buffer at 15 min intervals), after which cumulative concentrations of PE (10^{-9}−10^{-6} M) were added to the organ bath. The tissues were then washed over 60 min to restore basal tone before contracting to approximately 80% of the maximum PE-induced response. Once a stable response to PE was achieved,

cumulative concentration−response curves to the NO donors were constructed in the presence and absence of light (foil-covered organ baths versus illumination with the proper light source) and the sGC inhibitor ODQ (5 μM).[30]

cGMP Generation in Cultured Rat Aortic Smooth Muscle Cells. A7R5 vascular smooth muscle cells were purchased from the American Tissue Culture Collection (Manassas, VA). Cells were grown in Dulbecco's modified Eagle Medium (DMEM, Invitrogen, U.K.), supplemented with 10% New Zealand fetal bovine serum (FBS, TCS Biologicals, New Zealand), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, U.K., complete medium) in sterile conditions. The cell cultures were maintained at 37 °C in a humidified incubator containing 5% CO_2 in air and were passaged by trypsinization.

A7R5 cells were seeded in 6-well plates at a concentration of 5 $\times$ 10^5 cells/well in 5 mL of complete medium. After 24 h, the cells were washed with 5 mL of PBS and replenished with 5 mL of complete medium. The cells were preincubated with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX, 1 mM) for 15 min, followed by incubation with or without the ruthenium nitrosyl **1** (10^{-5}M) for 10 min. The cells were then exposed to low-intensity UV light (λ_{max} = 254 nm) for 3 min. The medium was aspirated and intracellular cGMP content was measured by ELISA (as described above).

Results

Compound Characterization/Properties. The ability of these complexes to release NO has already been established by monitoring light-dependent NO release with an NO electrode.[31] Both **1** ($[Ru(PaPy_3)(NO)]^{2+}$) and **2** ($[Mn(PaPy_3)(NO)]^{+}$) are soluble in solvents like water and acetonitrile, and both compounds rapidly release NO upon illumination. When exposed to low-intensity UV light (7 mW), the pseudo first-order rate of NO release (k_{NO}) in water for **1** is 8.0 $\pm$ 0.1 $\times$ 10^{-3} s^{-1} (data not shown). Compound **2** releases NO upon illumination with low-intensity visible light. In water, k_{NO} for this species is 5.60 $\pm$ 0.01 $\times$ 10^{-3} s^{-1} when a 60 W tungsten lamp was used as the light source.[27] The k_{NO} values depend both on the nature of the solvent and on the intensity of light. Following NO release, both complexes are converted to the corresponding aqua species (reactions 1 and 2). It should be noted that the two k_{NO} values cannot be compared directly because the intensities of the two light sources were different. The visible light source was 60 W, while the UV light source was only 7 mW. It is likely that with a UV source of equal intensity to the visible light source, compound **1** (the Ru-nitrosyl species) would be an equal if not better NO donor compared to the Mn complex.

Light-Dependent Activation of Purified sGC by the Metal Nitrosyls. One of the most sensitive and specific detectors for NO is sGC. Previous work indicates that, among all nitrogen oxides, NO appears to be the most potent activator of catalytic activity.[32] In the present study, the ability of two photolabile NO donors (**1** and **2**) to photoactivate purified human recombinant sGC was examined. As shown in Figure 1, both **1** and **2** elicit light-dependent activation of purified recombinant human sGC. In experiments with both nitrosyls, there was some variability in the activation of the enzyme in the "dark" controls, because it was difficult to completely eliminate all sources of light from the experiment. Regardless, a dramatic increase in activation was always observed when the experiment was exposed intentionally to the visible (in the case of **2**) or UV light (in the case of **1**) source. The effect of the NO donors was saturating at the higher concentrations, undoubtedly due to the fact that these experiments were performed under conditions of limiting enzyme.

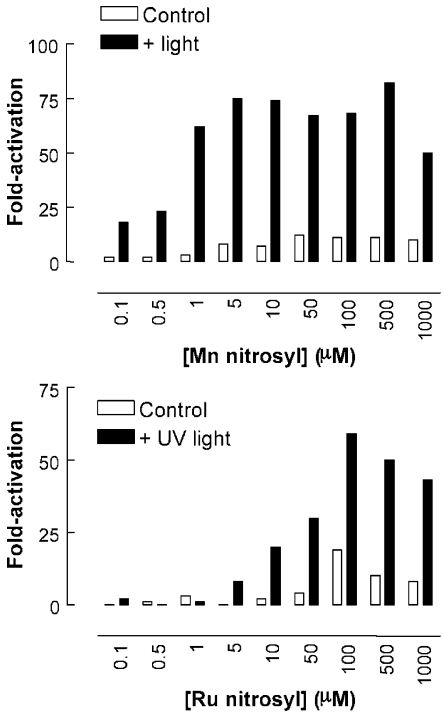


Figure 1. The effect of light-mediated NO release from $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**) on the activity of purified sGC. These results are representative of several experiments.

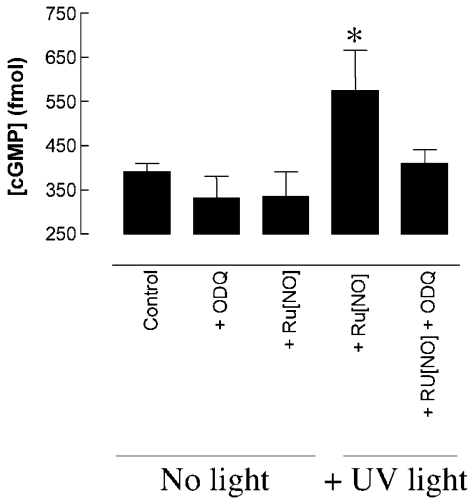


Figure 2. Light-dependent increase of cGMP levels in rat aortic smooth muscle cells (RASMC) by $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and the effect of the sGC inhibitor ODQ on the light-dependent effect.

Light-Dependent Increase in cGMP in Cultured Smooth Muscle Cells by 1. Cultured smooth muscle cells were exposed to the ruthenium nitrosyl **1** at concentrations of 0.1 and 1 mM. The cultures were then illuminated with a hand-held UV light for 5 min, followed by a 10-min incubation period in the absence of light. Cells were then harvested, and cGMP accumulation was determined as described above. As shown in Figure 2, there was a significant increase in cGMP in smooth muscle cells when the mixture of cells and **1** was exposed to UV light. In the absence of light, little or no increase in cGMP was observed. Moreover, the sGC inhibitor ODQ was found to inhibit the activation elicited by **1**.

Vasorelaxant Properties of the Nitrosyls. The ability of the nitrosyls to relax smooth muscle tissue upon exposure to light was assessed by using a tissue bath preparation. Rat thoracic aortic rings, precontracted with phenylephrine, were treated with

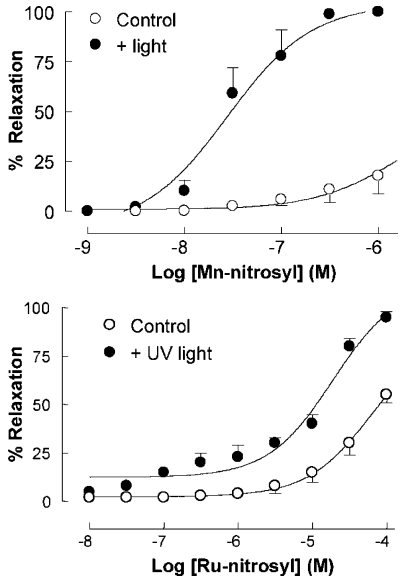


Figure 3. Vasorelaxant properties of $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**) in the presence and absence of light in rat thoracic aortic rings.

either **1** or **2** at varying concentrations. Light-dependent release of NO from the complexes was assessed by simply shining the appropriate light onto the tissue bath solution. Figure 3 (top panel) shows the dose- and UV-light-dependent vasorelaxant properties of the manganese nitrosyl **2**. In the absence of light, **2** had little vasorelaxant activity, even at concentrations as high as 1 μM. However, in the presence of light, **2** became a potent vasorelaxant with an EC_{50} of less than 50 nM. The ruthenium nitrosyl **1** also showed some vasorelaxant activity in the absence of light (up to concentrations of 1 μM) and with increased potency in the presence of UV light (Figure 3, bottom panel).

To determine whether the vasorelaxant activity of the photolabile NO donors **1** and **2** was due to the release of NO and the activation of sGC, the effect of the sGC inhibitor ODQ was examined. ODQ, an established inhibitor of sGC,[30,33] inhibits NO-mediated activation of sGC. As shown in Figure 4, relaxation from light-activation of either NO donor was inhibited by 5 μM ODQ, as were responses to the NO-donor SPER-NO (positive control). These results are consistent with the idea that the light-dependent vasorelaxation by **1** and **2** was a result of NO-mediated activation of sGC.

Discussion

Nitrovasodilator drugs have proved efficacious in the treatment of a number of cardiovascular disorders for almost 150 years and today are routinely used for treating angina and heart failure.[5] However, their therapeutic use is problematic for at least two reasons. First, the lipophilic and diffusible NO gas is not compartmentalized in the body but is freely distributed across all cells and tissues. While sGC represents the predominant intracellular target for physiological concentrations of NO, at higher levels, alternate targets become significant and, following chronic exposure to NO, the potential side effects are numerous and not well-characterized. Second, the archetypal NO donors used in current therapy, organic nitrates and nitroprusside, require bioactivation to release NO, possibly within the mitochondria.[34,35] This metabolic dependence results in tachyphylaxis or tolerance, necessitating administration of repeatedly higher concentrations of the drug (which will then exacerbate the sGC-independent actions of NO). Nonetheless, as the physiological and pathological roles of NO are further

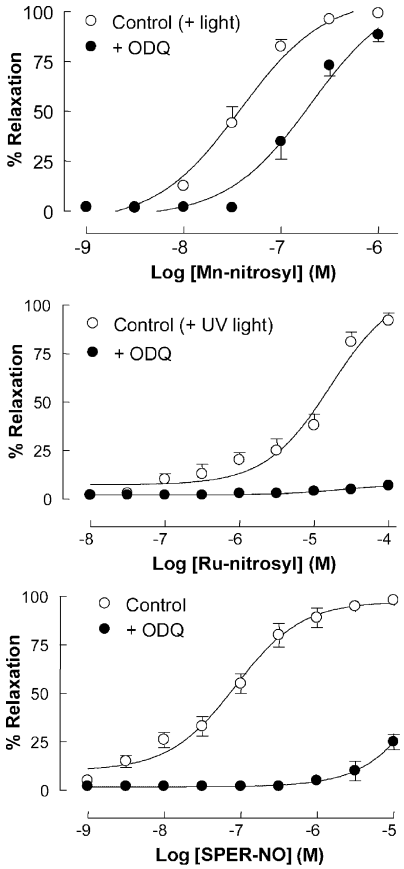


Figure 4. The effect of the sGC inhibitor ODQ on the light-induced vasorelaxant properties of $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**) in rat thoracic aortic rings. The NO donor SPER-NO was used as a positive control (**3**).

elucidated, it is likely that the therapeutic potential of NO donors (and inhibitors of NO biosynthesis and activity), will also expand. In such pursuit, the development and characterization of novel NO-donor drugs that might be able to avoid the problems of systemic distribution and tolerance is highly desirable.

The development of photoactive NO donors has the potential of novel application as a site-specific antitumor agent. Photodynamic therapy (PDT) involves the use of a photosensitizing agent (usually a porphyrin-based compound) that upon illumination with light of an appropriate wavelength leads to a cascade of molecular energy transfers and liberation of a cytotoxic species, often singlet oxygen.[36] The photosensitizers are designed to be preferentially taken-up by the diseased tissue/organ. This property in combination with selective light activation provides effective tumoricidal activity without damage to the surrounding tissue. Such a technique has been used in a number of conditions, including cancers of the esophagus, skin, bladder, lung, and brain. Because NO is known to possess potent antitumor activity,[37−39] photosensitizers that release NO may provide a novel route for effective PDT. However, the therapeutic potential of photolabile NO donors expands far beyond that of PDT. For example, topical application of NO donors to the skin may be beneficial in the treatment of the peripheral vasculopathies associated with Raynaud's phenomenon and diabetes mellitus. NO is also thought to play a key role in facilitating wound healing,[40] and therefore, application of photolabile NO donors to sites of injury, or surgical wounds, may well expedite recovery. Furthermore, NO is now known to be involved in the regulation of melanocyte activity[41] and UV-induced hyperpigmentation. As such, one can envisage a

potential photolabile NO-donor-based therapy in disease states associated with loss of pigmentation (e.g., vitiligo) and perhaps the more lucrative market of artificial tanning agents, although their long-term safety in terms of induction of melanomas would have to be established. Finally, administration of topical photolabile NO donors may be an improved therapy for patients who require GTN patches (e.g., nocturnal angina). Administration of GTN in such fashion still requires bioactivation and thus induces tolerance; consequently, patients must remove the patches intermittently. If a slow release photolabile NO donor could be administered topically and a long, slow release of NO were achieved (ideally by natural light), this may avoid tachyphylaxis and remain an effective anti-anginal agent, provided sufficient NO reached the systemic circulation.

In an attempt to address this therapeutic potential, we have synthesized a family of metal nitrosyls that release NO upon exposure to different, but specific, wavelengths of light.[24−29] The mechanism(s) of photorelease of NO from these nitrosyls is currently under investigation. A theoretical study by Richards[42] indicates strong correlation between the photolability of the corresponding iron species and the existence of low-energy transitions that promote an electron into the Fe−NO π^* antibonding molecular orbital. In the present study, we have characterized the in vitro biochemistry and pharmacology of two representative members of this novel photolabile NO-donor family, namely, $[Ru(PaPy_3)(NO)](BF_4)_2$ (**1**) and $[Mn(PaPy_3)(NO)]ClO_4$ (**2**). The best-characterized physiological target for NO is sGC. To assess the light-dependent release of NO from **1** and **2**, we have used purified human recombinant sGC. In the absence of light, addition of either **1** or **2** had little effect on sGC activity (as measured by cGMP formation), although a minor increase above basal turnover was observed in some experiments because it was logistically difficult to eliminate all light. However, upon illumination with light of an appropriate wavelength (UV light for **1** and visible light for **2**), there was a marked and concentration-dependent increase in sGC activity. Since NO is the only nitrogen oxide capable of activating sGC, our results demonstrate that both **1** and **2** are stable in the absence of light (the complexes in the solid state are relatively stable in the presence of light, only in solution do these complexes exhibit their light-sensitivity), but rapidly release NO upon exposure to light. Following this demonstration of NO-dependent activation of sGC in an isolated enzyme preparation, we then proceeded to look at the photosensitivity of these complexes in a more physiological environment, namely, in vascular smooth muscle cells in culture and in intact rings of rat aorta.

In the absence of light, there was little or no detectable cGMP generation in rat aortic smooth muscle (A7R5) cells in culture. Again, mirroring the stimulation of sGC activity in a purified enzyme preparation, in the presence of light, **1** elicited a concentration-dependent increase in cGMP content in the vascular smooth muscle cells (as mentioned previously, using the visible light activatable donor was problematic in these studies due to the high spurious ambient light in the cell culture experiment). Thus, a photolabile NO donor brings about activation of sGC in a cell-based system.

To study the biological activity of the photolabile NO donors in whole tissue, we have examined the vasorelaxant activity in rat thoracic aortic rings, a well-established system to assess the vasorelaxant potency of pharmacological agents. Here, both nitrosyls elicited a concentration-dependent relaxation of vessels precontracted with the α-adrenoceptor agonist phenylephrine when exposed to UV or visible light. Both NO donors were significantly less capable of causing relaxation of the tissue in the absence of light. This vasorelaxant activity was again mostly the result of NO release and sGC activation because the relaxations elicited by both **1** and **2** were attenuated in the presence of the sGC inhibitor ODQ. It is interesting that the inhibition of light induced effect associated with the Ru-nitrosyl species, **1**, by ODQ was significantly less than that observed for **2**. Currently, we do not have a good explanation for this, and this result may indicate another mechanism of vasorelaxation associated with **1**. Thus, in a whole tissue preparation, the same light-dependent NO release occurs from nitrosyls as was apparent in the purified enzyme preparation and the whole cell assay. Moreover, activation of sGC underlies the biological activity of these NO donors.

To be sure, the utility of these compounds, and related species, will be largely dependent on the pharmacologically relevant doses and the toxicity at those doses, as well as the source and wavelength of light. Although these issues have not yet been addressed, this study further establishes the "proof of principle" that light-dependent release of NO from complexes of this type is possible and can lead to biological activity (vasorelaxation, sGC activation, and cGMP generation). Clearly this is not the only report of photolabile NO generation (vide supra), and the utility of photolabile NO donors has been addressed previously.[10] This work extends the array of species capable of serving as light-activated NO donors and verifies the role of light in this regard. Moreover, these compounds may be particularly useful as therapeutic agents because the intensity of light required to elicit significant NO release is very low. Recently, photochemical release of NO from **2** incorporated into a sol−gel matrix has been observed.[31] Thus, use of such NO donors in biocompatible polymer matrices could be envisioned, possibly avoiding toxicological effects associated with the metals. Regardless, it is expected that future studies examining the detailed pharmacology/toxicology will be required to fully establish the utility of these, and related complexes, as potential therapeutic agents.

In summary, we have described the biochemistry and pharmacology of two novel photolabile metal nitrosyls. These NO donors are stable in the dark (both in solid state and in solution) and liberate NO only upon illumination with the appropriate wavelength of light. The liberation of NO is solely light-dependent, and no bioactivation is necessary. These compounds have a number of potential therapeutic applications, including photodynamic therapy, treatment of Raynaud's phenomenon, and peripheral vasculopathy associated with diabetes mellitus, contact dermatitis, wound repair, and vitiligo.

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