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Inorganica Chimica Acta 359 (2006) 2543–2549
Inorganica Chimica Acta
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Influence of ancillary ligand L in the nitric oxide photorelease by the $[Ru(L)(tpy)NO]^{3+}$ complex and its vasodilator activity based on visible light irradiation

Renata Galvão de Lima [a], Marilia Gama Sauaia [a], Daniella Bonaventura [b], Antonio Claudio Tedesco [a,b], Lusiane Maria Bendhack [b], Roberto Santana da Silva [a,b,*]

[a] *Departamento de Química da Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. dos Bandeirantes 3900, 14040-901 Ribeirão Preto, SP, Brazil*

[b] *Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, 14040-903 Ribeirão Preto, SP, Brazil*

Received 7 November 2005; received in revised form 2 February 2006; accepted 7 February 2006
Available online 6 March 2006

Abstract

The photochemical and pharmacological studies of the novel $[Ru(L)(tpy)NO]^{3+}$ L = bpy (2,2′-bipyridine), NH · NHq (quinonediimine) and NH_2.NH_2cat (*o*-phenylenediamine) were investigated in aqueous medium. The synthesized nitrosyl ruthenium complexes showed nitric oxide (NO) release under light irradiation at 355 nm for $[Ru(L)(tpy)NO]^{3+}$ complex with quantum yield of 0.14 ± 0.02, 0.47 ± 0.03 and 0.46 ± 0.02 mol Einstein^{-1} for L = bpy, NH · NHq and $NH_2 \cdot NH_2$cat, respectively, and 0.0065 ± 0.001 mol Einstein^{-1} for light irradiation at 532 nm for $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex. The photochemical pathway at 355 nm light irradiation was described as a multi-step mechanism, although at 532 nm it was better attributed to a photo-induced electron transfer. The vasorelaxation induced by NO release produced by light irradiation in visible region from physiological solution of $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex was evaluated and compared with sodium nitroprusside (SNP). The results showed very similar vasodilator power between both species.

Keywords: Nitrosyl ruthenium complexes; Vasodilation; Nitric oxide

1. Introduction

Nitric oxide (NO) is a radical molecule produced by many cells in the human body and has become recognized as a major effector molecule in a diverse array of physiological and pathological processes [1–5]. NO action has also been found in different cells and tissues in which it exhibits several different functions, such as neurotransmission, blood pressure control, inhibition of platelet aggregation, and immunological responses [6,7]. Due to the great biological importance of NO and also considering that many disease states are associated with a deficiency in nitric oxide, several species have been synthesized with the aim of developing nitric oxide-donor agents [8–14]. It might benefit from nitric oxide-enhancing medicines [15–17]. Among them, coordination compounds containing {M–NO^+} bond have been successfully employed as NO delivery agents by accessing the reduction potential of nitrosyl ligand [18–20] or by light stimulation [8–13,21,22]. Perhaps, a class of those nitrosyl species that have been most investigated is that concerning to the use of ruthenium(II) as metal center [21–24]. The ruthenium ion configurations are very useful due to their rich and versatile redox chemistry, in addition to their favorable thermodynamic and kinetic properties. Nitrosyl ruthenium complexes have usually been described as containing $\{Ru^{II}–NO^+\}$ bond, whose stability is pH-dependent, since it can react with

* Corresponding author.
E-mail address: silva@usp.br (R.S. da Silva).

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doi:10.1016/j.ica.2006.02.020

hydroxide ion to generate nitroruthenium(II) species [8–14,22,25]. The degree of electrophilicity of coordinated NO^+ can be systematically tuned through the modulation of ancillary ligand "L" in $[RuL_5NO]^{n+}$ type complex [26–28]. Two classes of nitrosyl ruthenium complexes, *cis*-$[Ru^{II}(bpy)_2L(NO^+)]^{n+}$ and *trans*-$[Ru^{II}L(NH_3)_4(NO^+)]^{3+}$, have been thoroughly studied because of their capability of releasing NO under light stimulation [8,10,23], which is one of our current interests [8–14]. The photochemical pathway for those complexes is produced by light irradiation on metal–ligand charge transfer band (MLCT), characterized as $(d_\pi Ru^{II})$–$\pi^*(NO^+)$ transition or by photo-induced electron transfer [8–14,22,25].

In an attempt to determine the controlling factors that allow managing the quantum yield of nitric oxide release, we have studied the photoreactivity of $[Ru(bpy)(tpy)NO](PF_6)_3$, $[Ru(NH \cdot NHq)(tpy)NO](PF_6)_3$ and $[Ru(NH_2 \cdot NH_2cat)(tpy)NO](PF_6)_3$ complexes, in which tpy is 2,2′:6′,2″-terpyridine, bpy is 2,2′-bipyridine, NH · NHq is quinonediimine and $NH_2 \cdot NH_2$cat is *o*-phenylenediamine (Fig. 1).

Some spectroscopic properties of $[RuL_4(benzoquinonediimine)]^{2+}$ complex have been described [31–33]. The species have shown great electron delocalization between the metal and the ligand that seems to be dependent on the energy, symmetries and overlap of valence metal and ligand orbital [34]. The non-innocent character of those quinone-related ligands permits to illustrate it in three different oxidation states, which are characterized as quinonediimine (NH · NHq), semiquinonediimine(NH · NHsq) and *o*-phenylenediamine ($NH_2.NH_2$cat) [35]. Based on this, our goal in this work was to describe the effect of this possible electron delocalization in some chemical and photochemical properties of $[Ru(bpy)(tpy)NO](PF_6)_3$, $[Ru(tpy)(NH \cdot NHq)NO](PF_6)_3$ and $[Ru(NH_2.NH_2cat)(tpy)NO](PF_6)_3$ complexes. In principle, the photolysis of both classes of complexes shows NO release by similar photochemical pathway. The possibility of using this type of complex as a NO delivery agent by light stimulation was also evaluated and interpreted by the pharmacological viewpoint.

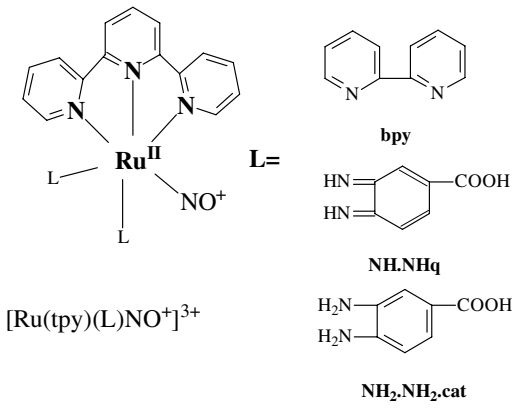


Fig. 1. Structure of nitrosyl ruthenium species.

2. Experimental

2.1. Apparatus

The ultraviolet–visible (UV–Vis) spectra were recorded on a Hitachi U-3501 and Genesys-2 apparatus from Spectronic. Infrared (IR) spectra were recorded on a protegé 460 series FT-IR spectrometer, using solid samples pressed in KBr pellets. The pH measurements were made using a 430 pH meter from Corning.

The photolysis of the complexes in a trifluoroacetate buffer solution at pH 2.01 and ionic strength of 0.1 M adjusted with $NaBF_4$ was performed using a laser flash photolysis apparatus consisting of a Continuum Q-switched Nd:YAG laser (Continuum, Santa Clara, CA) with excitation provided by a third (355 nm) and second (532 nm) harmonic. The pulse length was 8 ns, the beam diameter incident on the sample was 6 mm, and the repetition rate was 10 Hz. The pulse energy was typically 10 mJ/pulse measured with a Field Master power-meter with an L-30 V head. NO releasing was detected and measured with an ISO-NOP NO meter from World Precision Instruments, which directly detects NO concentration by an amperometric technique. The sensitivity of this apparatus ranges from 1 nM to 20 μM, with a 2-mm sensor, which directly detects NO concentration by an amperometric technique. The sensor output was recorded with an IBM-PC computer linked to a DUO-18 acquisition board from WPI.

2.2. Chemicals and reagents

$RuCl_3 \cdot nH_2O$, 2,2′:6′,2″-tpyridine, 2,2′-bipyridine, 3,4-benzoquinonediiminebenzoic acid, and 1,10′-phenanthroline, and Reinecke's salt $\{(NH_4)Cr(NH_3)_2(NCS)_4\ H_2O\}$ were purchased as high purity reagents from Aldrich Chemicals and were used as supplied. Potassium ferrioxalate, $\{K_3[Fe(C_2O_4)_3] \cdot 3H_2O\}$, was purchased from Fisher Scientific Co. Doubly distilled H_2O was used for all experiments.

The recrystallized complex salts [RuCl(bpy)(tpy)]Cl and $[Ru(bpy)(tpy)NO](PF_6)_3$ were prepared as previously published [36,37].

The [RuCl(NH · NHq)(tpy)]Cl complex was synthesized by a similar procedure described for [RuCl(bpy)(tpy)]Cl complex, however using 3,4-benzoquinonediiminebenzoic acid as ligand instead of 2,2′-bipyridine. The obtained ruthenium species was purified through a silica-gel column (70–230 mesh/Merck) by elution with methanol. A red solution fraction was separated and the solid was recovered by rotary evaporation and used in the synthesis of $[Ru(NH \cdot NHq)(tpy)NO](PF_6)_3$ complex. Yield = 20.0%.

The $[Ru(NH \cdot NHq)(tpy)NO](PF_6)_3$ complex was prepared by mixing 0.038 g of [RuCl(NH · NHq)(tpy)]Cl salt (0.069 mmol) previously dissolved in water (30 ml) under argon atmosphere with 0.024 g of $NaNO_2$ (0.34 mmol) and refluxed for 1 h. Two milliliters of HPF_6 was added

to the stirred solution. The resulting orange precipitate was collected by filtration, washed with diethyl ether and stored under vacuum in the dark. Typical yield for [Ru(NH · NHq)(tpy)NO](PF$_6$)$_3$ was 87.0 %. *Anal.* Calc. for $C_{22}H_{17}N_6O_3P_3F_{18}Ru$: C, 27.82; H, 1.89; N, 8.83. Found: C, 27.90; H, 1.99; N, 8.99%.

The [Ru(NH$_2$.NH$_2$cat)(tpy)NO](PF$_6$)$_3$ complex was prepared by mixing 0.023 g of [RuCl(NH · NHq)(tpy)]Cl salt (0.041 mmol) previously dissolved in water (50 ml) under argon atmosphere with 0.014 g of $NaNO_2$ (0.21 mmol). The mixture was put in a microwave with 30% potency during 2 min. Two milliliters of HPF_6 was added to the stirred solution. The resulting orange precipitate was collected by filtration, washed with diethyl ether and stored under vacuum in the dark. Typical yield for [Ru(NH$_2$ · NH$_2$cat)(tpy)NO](PF$_6$)$_3$ was 75.0%. *Anal.* Calc. for $C_{22}H_{17}N_6O_3P_3F_{18}Ru$: C, 27.76; H, 2.00; N, 8.83. Found: C, 27.51; H, 2.00; N, 8.82%.

2.3. Laser flash photolysis experiments

Photolysis of the nitrosyl ruthenium species (1.0×10^{-4}–1.0×10^{-5} M) was performed in trifluoroacetate buffer solution at pH 2.01 and the ionic strength of 0.1 M was adjusted with $NaBF_4$. A sample (3.0 ml) in a quartz 1-cm square cuvette with a magnetic stirring bar was routinely thermostated at 25.0 ± 0.1 °C. The sample solution was stirred continuously and was irradiated for a defined time period.

Two types of analyses were carried out during the flash photolysis experiments. First, the UV–Vis spectrum of the sample was recorded after each irradiated period. Second, the NO measurements were made with the selective electrode (NOmeter) into the cuvette, but positioned outside the light path to avoid any photoelectric interference.

2.4. NOmeter calibration

The calibration curve of the selective electrode was constructed by several dilutions of a known volume of a saturated nitric oxide solution in 10.0 ml of a previously degassed trifluoroacetate buffer solution (pH = 2.01), to which the NOmeter electrode was adapted. The current value in nA was recorded for each added volume. NO concentration was calculated according to the reported NO molar fraction solubility (2.1×10^{-3} M at 25 ± 0.1 °C) [38].

Nitric oxide gas was produced by Cu^0 dyed in HNO_3 solution (50%) and was passed through a 5 M KOH solution to remove any trace of nitrite species. Nitric oxide stock solution was prepared by degassing 10 ml of distilled water in which the NO gas was bubbled during 30 min.

2.5. Quantum yield measurements

Light intensities were determined before each photolysis experiments by chemical actinometry procedure. The actinometers used were potassium ferrioxalate to $\lambda_{irr} = 355$ nm and Reinecke's salt to $\lambda_{irr} = 532$ nm [39,40]. An average was obtained from the numeric values of light intensity, which were calculated according to the literature [41].

NO quantum yields (ϕ_t) were calculated based on NO concentrations, obtained by NO meter measurement. The calculated values were plotted versus t. These plots were linear, with a negative slope, for the first 40% of the reaction. The extrapolated quantum yield at $t = 0$ (y intercept) was taken as ϕ_{NO} for the photolabilization of NO from [Ru(L)(tpy)NO]$^{3+}$compounds. Evaluation of ϕ_{NO} at $t = 0$ eliminates possible complications, resulting from primary products photoreactions [41,42]. All the reported data are the average of three independent experiments, and the error is the standard deviation.

2.6. Pharmacological experimental protocols

Vessel preparation was done as previously described [12].

2.6.1. Experimental protocols

2.6.1.1. Relaxant effect of [Ru(NH · NHq)(tpy)NO]$^{3+}$ after pre-contraction with norepinephrine, phenylephrine and prostaglandin $F_{2\alpha}$. To examine whether [Ru(NH · NHq)(tpy)NO]$^{3+}$ induces smooth muscle cell relaxation, aortic rings were pre-contracted with 0.1 μM norepinephrine (NOR), 0.1 μM phenylephrine (Phe) and 3 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the contraction had reached, a plateau [Ru(NH · NHq)(tpy)NO]$^{3+}$ (1.0×10^{-10}–3.0×10^{-4} M) was cumulatively added.

2.6.1.2. Time-course for the relaxation induced by [Ru(NH · NHq)(tpy)NO]$^{3+}$. [Ru(NH · NHq)(tpy)NO$^+$]$^{3+}$ (1.0×10^{-4} M) was added to the organ chamber when a stable contraction in response to 1×10^{-5} M phenylephrine was achieved. Time-course for relaxation induced by this compound was evaluated and a similar protocol was followed for sodium nitroprusside (SNP) (3.0×10^{-5} M).

2.6.1.3. Data analysis. Data are expressed as means ± SEM. In each set of experiments, n indicates the number of rats studied. The maximum relaxant effect (E_{max}) was considered as the maximal amplitude response reached in the concentration-effect curves for the compounds. The concentrations of the agents that produced the half-maximal relaxation amplitude (EC_{50}) were determined after logit transformation of the normalized concentration-response curves and were reported as the negative logarithm (pEC_{50}) of the mean of individual values for each tissue using the GraphPad Prism version 3.0 (GraphPad Software Corporation San Diego, CA).

3. Results and discussion

The [Ru(NH · NHq)(tpy)NO](PF$_6$)$_3$ complex described in this work was prepared by a similar procedure previously published for [Ru(bpy)(tpy)NO](PF$_6$)$_3$ [36]. The [Ru(NH$_2$ · NH$_2$q)(tpy)NO](PF$_6$)$_3$ was achieved using

microwave as a synthetic method. It is likely that the short time used in that reaction was responsible for the isolation of the quinoneammine ruthenium complex. Those nitrosyl ruthenium complexes were characterized by elemental analysis and conventional spectroscopic techniques. Some data concerning to UV–Vis and IR spectra results of the nitrosyl ruthenium complexes and related species are shown in Table 1.

The $[Ru(L)(tpy)NO]^{3+}$ and $[Ru(X)(L)(tpy)]^{n+}$ complexes, in which L = bpy or benzoquinonediimine ligands and X = Cl, show intraligand bands in the UV region that are attributed to $\pi \rightarrow \pi^*$ transitions of the unsaturated ligands in comparison to the electronic spectrum of free ligand. The $[Ru(H_2O)(NH \cdot NHq)(tpy)]^{2+}$ was obtained in situ by the reduction of $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ with zinc amalgam, as previously described for similar nitrosyl ruthenium complexes [8–13]. All the studied nitrosyl ruthenium species also showed a shoulder on the 300–400 nm region (Table 1) that is not observed on the electronic spectrum of $[RuCl(bpy)(tpy)]^{+}$ and $[Ru(X)(NH \cdot NHq)(tpy)]^{n+}$. Based on this and by comparison to the described electronic spectrum of similar nitrosyl ruthenium species [8,10,26–28], we tentatively assigned this band as metal ligand charge transfer (MLCT) due to $d_{\pi}(Ru^{II}) \rightarrow \pi^*(NO)$ transition. For the $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex, we found, on its electronic spectrum, a weak band at 510 nm that was attributed to the $d\pi(Ru^{II}) \rightarrow \pi^*(NH \cdot NHq)$ transition (Fig. 2) by comparison to the electronic spectrum of $[Ru(X)(NH \cdot NHq)(tpy)]^{n+}$ ($X = H_2O$ or Cl^-).

The IR spectrum exhibits a strong vibrational peak at 1850–1950 cm^{-1} region ascribed to ν_{NO} stretching. The cationic character of NO^+ of coordinated nitric oxide is related to the extent of the $d_{\pi}(Ru^{II})$–NO^+ interaction, which is affected by the binding metal center with ancillary ligands in the nitrosyl ruthenium species. Thus, we expect that a change of the L ligand on $[Ru(L)(tpy)NO]^{3+}$ complex should modify the ruthenium nitrosyl interaction and consequently the ν_{NO} stretching, as observed for several different classes of nitrosyl ruthenium complexes [10,26–30,43].

The observed lowest ν_{NO} stretching energy for $[Ru(tpy)(NH_2 \cdot NH_2cat)(NO)]^{3+}$ complex, in comparison to the other described nitrosyl terpyridine ruthenium complex, can roughly be used to the attribution of strengthening $\{Ru^{II}–NO^+\}$ interaction or may be due to the effect occasioned by the co-ligand *trans* to NO^+, as observed for $[RuCl_2(tpy)NO]Cl$ [18].

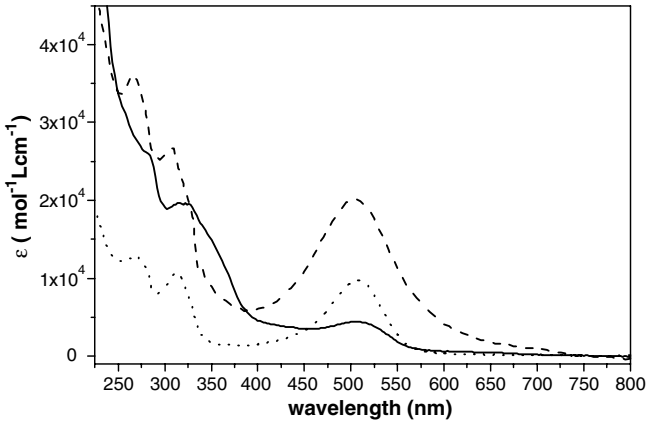


Fig. 2. Electronic spectra of $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex at (solid line), $[Ru(H_2O)(NH \cdot NHq)(tpy)]^{2+}$ (dash line) and $[RuCl(NH \cdot NHq)(tpy)]^{+}$ (dot line) complexes in HCl 0.1 M.

The photolysis at 355 nm of an aqueous solution containing $[Ru(bpy)(tpy)NO]^{3+}$, $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ and $[Ru(NH_2 \cdot NH_2cat)(tpy)NO]^{3+}$ was monitored by UV–Vis spectra. The UV–Vis absorption spectral change containing $[Ru(bpy)(tpy)NO]^{3+}$, during irradiation is shown in Fig. 3. The electronic spectrum of the photoproduct is very similar to the synthesized $[Ru^{II}(H_2O)(bpy)(tpy)]^{2+}$ complex [36]. Assuming that the metal ligand

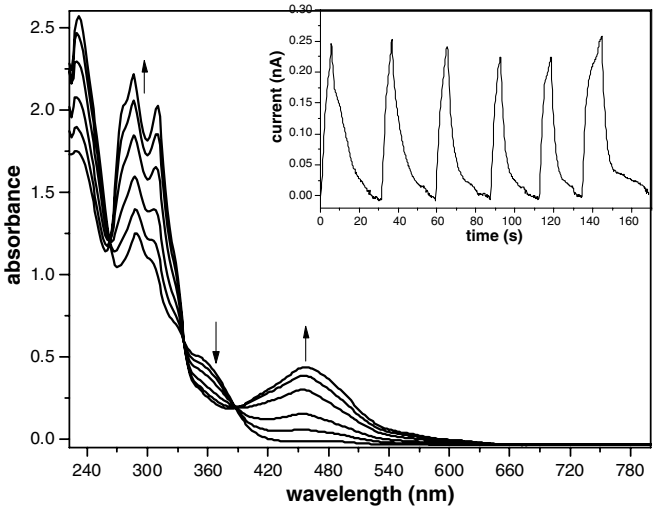


Fig. 3. Electronic spectra of $[Ru(bpy)(tpy)NO]^{3+}$ complex, pH 2.01, during flash-photolysis at 355 nm. [Complex] = 5.87×10^{-5} M. Inset: Chronoamperogram of NO release by photolysis of $[Ru(bpy)(tpy)NO]^{3+}$ complex. [Complex] = 1.00×10^{-4} M.

Table 1
UV–Vis and infrared data of $[Ru(L)(tpy)NO]^{3+}$ complex, with L = bipyridine (bpy), quinonediimine ($NH \cdot NHq$) and *o*-phenylenediamine ($NH_2 \cdot NH_2cat$) and related species

Complex	λ (nm) (log ε)[a]	νNO (cm^{-1})[b]
$[Ru(bpy)(tpy)NO](PF_6)_3$	230 (4.63); 277 (4.25); 288 (4.32); 305 (4.24); 332 (4.02); 358 (3.92); 480 (2.84)	1944
$[Ru(NH_2 \cdot NH_2cat)(tpy)NO](PF_6)_3$	288 (4.21); 325 (4.11); 355 (3.98)	1874
$[Ru(NH \cdot NHq)(tpy)NO](PF_6)_3$	285 (4.39); 324 (4.27); 358 (4.15); 510 (3.65)	1888
$[RuCl(tpy)(NH \cdot NHq)]Cl$	266 (4.07); 278 (4.05); 312 (4.02); 328 (3.89); 502 (3.98)	

[a] HCl 0.1 M.
[b] KBr pellets.

charge transfer between Ru(II) and NO^+ occurs at 350 nm for $[Ru^{II}(tpy)(L)NO^+]^{3+}$ complex, the irradiation in this region could easily generate $[Ru^{III}(bpy)(tpy)NO^0]^{3+}$ complex at excited state. Since the transient is formally Ru^{III}–NO^0, the substitution reaction is expected to occur considering many of the reports that present the NO^0 species as a labile ligand for ruthenium(III) complex [8–10,14]. However, the formation of an aqueous ruthenium(II) species should be possible presumably by accessing a lowest energy ligand field state, during the TCML state deactivation.

The photoreactivity of the nitrosyl complexes was also accompanied by in situ NO detection. The signal recorded by the NO sensor rose quickly when photolysis was initiated, then decreased when the light was turned off owing to NO consumption via various pathways, especially auto-oxidation [44] (inset, Fig. 3). Considering that the photolysis at 355 nm is due to light irradiation on the MLCT band occasioned by $d_\pi(Ru^{II}) \rightarrow \pi^*(NO)$ transition, the $[Ru(H_2O)(bpy)(tpy)]^{3+}$ complex should be expected as one of the photoproducts. Similar behavior was also observed for all the studied complexes.

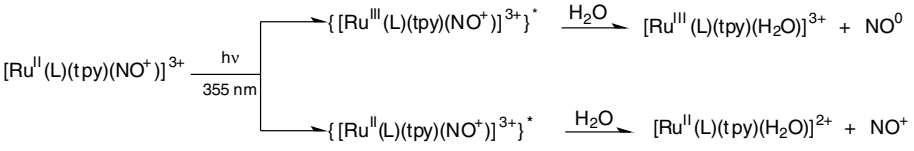
Based on the spectroscopic results and by the NO measurement, we could infer the photochemical pathway as a multiple photochemical process as described on Scheme 1.

The quantum yields observed for NO release from $[Ru(L)(tpy)NO]^{3+}$ complexes, L = bpy, NH · NHq and $NH_2 \cdot NH_2$cat, obtained by 355 nm light irradiation were 0.14 ± 0.02, 0.47 ± 0.03 and 0.46 ± 0.02 mol Einstein^{-1}, respectively. The highest NO quantum yield found for the derivative quinoneammine complexes can be taken as indication for the formation of a large amount of $\{Ru^{III}$–$NO^0\}$ fragment at excited state in comparison to the bipyridine ruthenium complex. Perhaps, the delocalized electron density between the quinoneammine, nitrosyl and the metal ion facilitates the incidence of $\{[Ru^{III}(NH \cdot NHq)$-(tpy)-$NO^0]^{3+}\}^*$, which is accentuated when the derivative quinoneammine ligand is present. The [Ru(NH · NHq)-(tpy)- $NO]^{3+}$complex was also submitted to light irradiation at 532 nm, since the aqueous solution of this species shows a band in the visible region. The electronic spectrum during light irradiation shows little decreasing of the intensity of MLCT band attributed to $d_\pi(Ru^{II}) \rightarrow \pi^*$(N-H · NHq) transition. The observed NO quantum yield obtained by the NO-selective sensor measurement was 0.0065 ± 0.001 mol Einstein^{-1}. This value is consistent with the expected quantum yield for NO release obtained by photo-induced electron transfer for some nitrosyl ruthenium species [11,14]. Assuming that the light irradiation at 532 nm is mainly attributed to the photochemical process centered on the $d_\pi(Ru^{II}) \rightarrow \pi^*(NH \cdot NHq)$ transition, it is licit to suppose that the photochemical pathway of this process involves a photo-induced electron transfer by the former NH · NHsemiquinone from $\{Ru^{III}$–(NH · NHsemiquinone)$\}^*$ to the nitrosyl ligand, as described on Scheme 2. Considering that semiquinone is a powerful reducing agent, the proposed photochemical mechanism should involve the production of $\{Ru^{III}$–(NH · NHq)$\}$ fragment.

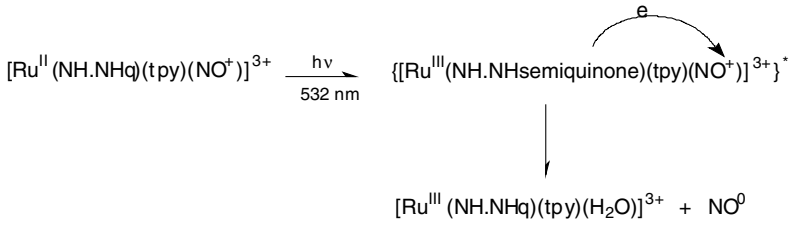
3.1. Pharmacological experiment

The $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex shows kinetic stability on physiological solution, which allowed us to use this compound on vasorelaxation studies. The relaxation induced by this compound was concentration-dependent in denuded rat aortas pre-contracted with 1.0×10^{-7} M norepinephrine or 1.0×10^{-7} M phenylephrine or 3.0×10^{-6} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The results showed that the relaxation induced by [Ru(NH · NHq)(tpy)NO]$^{3+}$ complex was the same for all contractive agents used (NOR: $E_{max} = 102.38 \pm 0.38\%$, $pEC_{50} = 6.47 \pm 0.13$, $n = 6$; Phe: $E_{max} = 103.48 \pm 1.03\%$, $pEC_{50} = 6.61 \pm 0.09$, $n = 8$; $PGF_{2\alpha}$: $E_{max} = 102.24 \pm 0.42\%$, $pEC_{50} = 6.48 \pm 0.08$, $n = 6$).

In fact, the production of NO has been taken as a consequence for the observed vasodilatation [45]. We found that, in physiological medium, NO is produced by an external stimulation of a nitrosyl ruthenium complex [9,10,12] mainly by the action of an aqueous solution containing



Scheme 1.



Scheme 2.

noradrenaline, a known reducing agent. Considering that the $PGF_{2\alpha}$ is not a reducing agent, as established for noradrenaline [20] or phenylephrine, and taking into account that the vasorelaxation curve between the plots is very similar (Fig. 4), we can infer that $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex induced vasorelaxation by an intracellular mechanism, as previously attributed for SNP [45].

The study of time-course for the relaxation induced by $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ showed that the time to reach maximum relaxation under dark is around 3500 s (Fig. 5), although under visible light irradiation it took 255 s, which is comparable to SNP (242 s, $n = 5$).

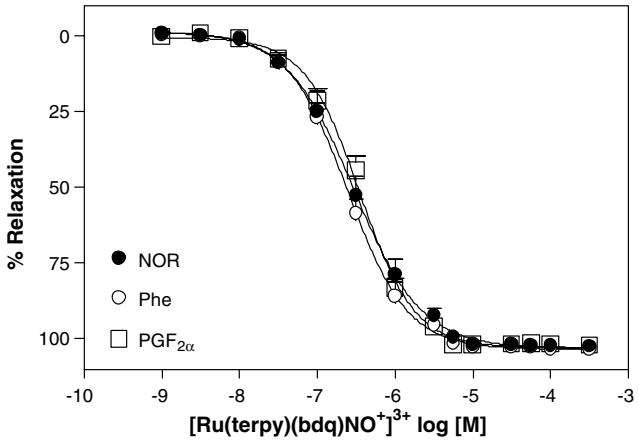


Fig. 4. Relaxation induced by $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex in rat aorta pre-contracted with phenylephrine (1.0×10^{-7} M), norepinephrine (1.0×10^{-7} M) or Prostaglandin $F_{2\alpha}$ (3.0×10^{-6} M). Relaxation responses are expressed as percent reversal of the contractive agent-induced contraction. Data are means ± SEM of n experiments performed on preparations obtained from different animals.

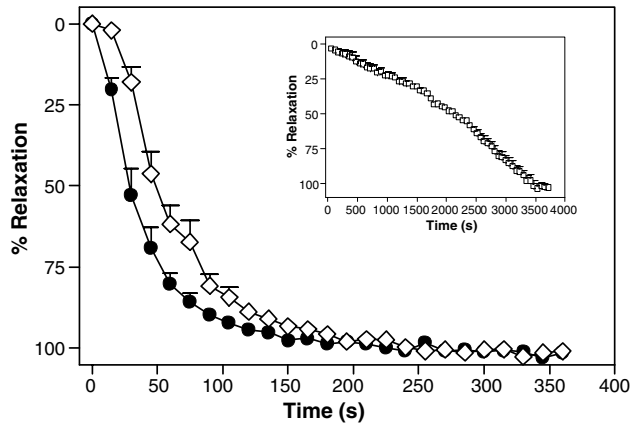


Fig. 5. Time-course for $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex-induced relaxation. Denuded thoracic aortic rings were pre-contracted with phenylephrine (1.0×10^{-7} M) after addition of 1.0×10^{-4} M of complex in buffer solution, pH 7.40, under visible light irradiation (◇) and NPS (●). Data are means ± SEM of n experiments performed on preparations obtained from different animals. Inset: Time-course for $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex-induced relaxation. Denuded thoracic aortic rings were pre-contracted with phenylephrine (1.0×10^{-7} M) after addition of 1.0×10^{-4} M of complex in buffer solution, pH 7.40, in the dark.

4. Conclusion

The photolysis of $[Ru(L)(tpy)NO]^{3+}$ produced NO with quantum yield dependent on the L ligand. Apparently, the electron delocalization existent between ruthenium and benzoquinonediimine ligand induces an increase of back bonding in $\{Ru^{II}–NO^{+}\}$ interaction, thus producing high NO quantum yield values when submitted to light irradiation in ultraviolet region, in comparison to the complex containing bipyridine ligand.

The observed vasorelaxation for the physiological $[Ru(NH \cdot NHq)(tpy)NO]^{3+}$ complex solution showed that this complex can be a powerful NO delivery agent and may be a good candidate to be used in clinical therapy.

Acknowledgments

This work was supported by grants from FAPESP, CNPq and Pronex.

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