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ScienceDirect
Vascular Pharmacology 46 (2007) 215–222
Vascular Pharmacology
www.elsevier.com/locate/vph

Comparison of the mechanisms underlying the relaxation induced by two nitric oxide donors: Sodium nitroprusside and a new ruthenium complex

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Received 21 April 2006; received in revised form 10 July 2006; accepted 3 October 2006

Abstract

We studied the mechanisms involved in the relaxation induced by nitric oxide (NO) donors, ruthenium complex ($[Ru(terpy)(bdq)NO^+]^{3+}$-TERPY) and sodium nitroprusside (SNP) in denuded rat aorta. Both NO donors induced vascular relaxation independent of the agonist used in the pre-contraction. $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP activated guanylyl cyclase (GC) and K^+ channels. The production of cGMP induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ — was higher than that obtained with SNP. The combination of GC inhibitor with K^+channels blocker almost abolished the relaxation induced by the NO donors. The extracellular NO scavenger oxyhemoglobin reduced the potency without changing the maximum effect (Emax) of both NO donors. By using specific NO species scavengers, hydroxocobalamin and L-cysteine, we have identified the contribution of free radical NO ($NO^\bullet$) and nytroxil anion (NO^-), respectively, to the rat aorta relaxation induced by both NO donors. The selective scavengers for $NO^\bullet$ and NO^- reduced the potency but not the Emax of $[Ru(terpy)(bdq)NO^+]^{3+}$. However, the NO^- scavenger had no effect on the relaxation induced by SNP and $NO^\bullet$ scavenger reduced only the potency to SNP. The inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase reduced only the potency of SNP without effect on the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$. Our results demonstrate that both NO donors induce relaxation by activating the GC and K^+ channels. The $NO^\bullet$ is the unique NO specie involved in the SNP-relaxation. On the other hand, the relaxant effect of $[Ru(terpy)(bdq)NO^+]^{3+}$ involves both $NO^\bullet$ and NO^-, that produce higher concentration of cGMP. The inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase reduces the relaxation induced by SNP but it did not alter the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$.

Keywords: Nitric oxide donor; Nitric oxide species; Guanylyl cyclase; K^+ channels and vasorelaxation

1. Introduction

In recent years, nitric oxide (NO) has been shown to be involved in many important biological events. Among its diverse functions, NO has been implicated in vascular smooth muscle relaxation (Moncada et al., 1986a,b; Ignarro, 1989a,b). The predominant signaling pathway responsible for the vascular relaxation induced by NO appears to involve the activation of soluble guanylyl cyclase and the subsequent accumulation of cGMP and activation of protein kinase G (Lohse et al., 1998). However, several authors have presented evidence suggesting that vascular relaxation to NO may occur through a cGMP-independent stimulation of smooth muscle by the direct activation of potassium (K^+) channels (Bolotina et al., 1994; Plane et al., 1996; Mistry and Garland, 1998; Homer and Wanstall, 2000).

Nitric oxide donors are pharmacologically active substances that in-vivo or in-vitro release NO. The majority of them have a nitroso functional group within the molecule. In the NO donor, sodium nitroprusside (SNP), a molecule of NO is coordinated to iron metal forming the square bipyramidal complex with five cyanide anions (CN^-). Interaction of SNP with reducing agent leads to the formation of NO (Bates et al., 1991). In the vascular system, NO release by SNP requires the presence of vascular

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doi:10.1016/j.vph.2006.10.002

tissue (Bates et al., 1991; Marks et al., 1995). It is likely that the release of NO by vascular tissues including smooth muscle cell membranes serves this reducing function. NO formation from SNP is accompanied by cyanide release (Bates et al., 1991) and it can be toxic to the vascular cells. In another study, the authors reported that SNP could be involved in the formation of superoxide anions (O_2^-) (Lamarque and Whittle, 1995). Superoxide reacts with NO released from SNP and forms peroxynitrite ($ONOO^-$) that is a damaging oxidant, which could be responsible for general and permanent tissue damage (Villa et al., 1994) and apoptosis (Estevez et al., 1995). Based on these toxic effects of SNP, some metallonitrosyl complexes have been used as NO deliver agents (Wang et al., 2000; Sauaia et al., 2003; Oliveira et al., 2004). Nitrosyl ruthenium complexes are attractive because of their thermal stability and their stability in physiological pH (Sauaia et al., 2003; Oliveira et al., 2004; de Lima et al., 2006). We have explored the potential of nitro ruthenium complex as NO deliver agent in physiological conditions (Bonaventura et al., 2004, 2005). Nitrosilo–terpyridine–phenylene–diamine ruthenium(II) ion complex ($[Ru(terpy)(bdq)NO^+]^{3+}$) is another nitro ruthenium complex that was chemically characterized, which vascular relaxation effect is accelerated in the presence of light (de Lima et al., 2006). Therefore, the current study was designed to characterize the type of NO released and to determine the cellular mechanisms that are activated by this nitro ruthenium complex to induce rat aorta relaxation. The effects of the new complex $[Ru(terpy)(bdq)NO^+]^{3+}$ were compared to those induced by the classical NO donor SNP.

2. Materials and methods

2.1. Vessel preparation

Male Wistar rats (400–450 g) were killed by decapitation and all the procedures are in accordance with the Ethical Animal Committee of the University of São Paulo, Brazil. The thoracic aorta was quickly removed, dissected free, and cut into 4 mm long rings. In order to avoid the interference of the endothelium factors, in the present study we investigated the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP in endothelium-denuded arteries. The endothelium was mechanically removed by gently rolling the lumen of the vessel on a thin wire. The aortic rings were placed between two stainless-steel stirrups and connected to an isometric force transducer (Letica Scientific Instruments; Barcelona—Spain) to measure tension in the vessels. The rings were placed in a 10 mL organ chamber containing Krebs solution with the following composition (mmol/L): NaCl 130, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 14.9, glucose 5.5, $CaCl_2$ 1.6. The solution was maintained at pH 7.4 gassed with 95% O_2 and 5% CO_2 at 37 °C. The rings were initially stretched to a basal tension of 1.5 g before allowing them to equilibrate for 60 min in the bath fluid, which was changed every 15–20 min. Endothelial integrity was qualitatively assessed by the degree of relaxation caused by acetylcholine (1 μmol/L) in the presence of contractile tone induced by phenylephrine (0.1 μmol/L). Since our studies required endothelium-denuded aortas, the rings were discarded if there was any degree of relaxation.

2.2. Experimental protocols

2.2.1. Relaxant effect of $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP after pre-contraction with norepinephrine, phenylephrine or prostaglandin $F_{2\alpha}$

To examine if the agent used to induce pre-contraction modulates the relaxation induced by the nitric oxide donors, the aortic rings were pre-contracted with the EC_{50} of the following contractile agents: 0.1 μmol/L norepinephrine, 0.1 μmol/L phenylephrine or 3 μmol/L prostaglandin $F_{2\alpha}$. When the contraction had reached a plateau, $[Ru(terpy)(bdq)NO^+]^{3+}$ (1 nmol/L to 30 μmol/L) or SNP (0.1 nmol/L to 0.3 μmol/L) were cumulatively added.

2.2.2. Effect of oxyhemoglobin on the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP

The oxidation of hemoglobin was performed as previously described (Martin et al., 1985). Oxyhemoglobin (HbO_2 10 μmol/L), an extracellular NO scavenger, was added 30 min before the addition of the contractile agonist chosen (0.1 μmol/L phenylephrine). Subsequently, cumulative concentration–response curves to $[Ru(terpy)(bdq)NO^+]^{3+}$ (1 nmol/L to 300 μmol/L) or SNP (0.1 nmol/L to 10 μmol/L) were obtained.

2.2.3. Effect of the NO scavengers hydroxocobalamin and L-cysteine on the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP

To investigate which NO form is involved in the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP, hydroxocobalamin (0.1 mmol/L), a free radical nitric oxide ($NO^\bullet$) scavenger or L-cysteine (1 mmol/L), a nitroxyl anion scavenger, were incubated for 30 min before the addition of 0.1 μmol/L phenylephrine. Subsequently, cumulative concentration–response curves to $[Ru(terpy)(bdq)NO^+]^{3+}$ (1 nmol/L to 300 μmol/L) or SNP (0.1 nmol/L to 1 μmol/L) were obtained.

2.2.4. Effect of guanylyl cyclase inhibitor and potassium channels blocker in the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ or SNP

To examine the contribution of guanylyl cyclase or potassium channels to the relaxation induced by $[Ru(terpy)(bdq)NO^+]^{3+}$ and SNP, ODQ (1 μmol/L), a selective guanylyl cyclase inhibitor, or tetraethylammonium (TEA 1 mmol/L), a non-selective potassium channel blocker, were incubated for 30 min before the addition of phenylephrine (0.1 μmol/L). Subsequently, cumulative concentration-response curves for $[Ru(terpy)(bdq)NO^+]^{3+}$(1 nmol/L to 300 μmol/L) or SNP (0.1 nmol/L–100 μmol/L) were obtained.

To verify whether the potassium channels are directly activated by NO released from the NO donors or if there is a pathway dependent on cGMP production, we have incubated the aorta rings with the combination of ODQ (1 μmol/L) and TEA (1 mmol/L) together. ODQ and TEA were incubated 30 min before the addition of phenylephrine (0.1 μmol/L) and