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Mechanisms underlying the hypotensive and vasodilator effects of Ru(terpy)(bdq)NO]$^{3+}$, a nitric oxide donor, differ between normotensive and spontaneously hypertensive rats

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ABSTRACT

The endothelium impairs the vasodilator effect of Ru(terpy)(bdq)NO]$^{3+}$ (TERPY) in Wistar rat aortas. We hypothesized that endothelial dysfunction could modulate TERPY's effect in spontaneously hypertensive rats. The present study investigated the role of the endothelium in the hypotensive and vasodilator effects of TERPY in spontaneously hypertensive rats. We observed a higher hypotensive effect of TERPY in spontaneously hypertensive than in Wistar rats. L-N^G-Nitroarginine methyl ester, a nitric oxide synthase inhibitor, increased TERPY's hypotensive effect in Wistar but not in spontaneously hypertensive rats. TERPY induced a concentration-dependent vasodilator effect in aortas of both rat models. Endothelium removal or L-NAME increased TERPY's potency in Wistar rat aortas; this effect was decreased in spontaneously hypertensive rats. TERPY increased nitric oxide level in spontaneously hypertensive rat endothelial cells; this increase was abolished in the presence of L-NAME. In contrast, this effect was increased in Wistar rats. TERPY, with or without L-NAME, decreased levels of reactive oxygen species in spontaneously hypertensive rat endothelial cells. However, it increased these levels in Wistar rats. TERPY reduced aortic endothelial nitric oxide synthase expression in Wistar rats, but did not alter its expression in spontaneously hypertensive rats. In conclusion, different mechanisms underlie the hypotensive and vasodilator effects of TERPY in these two rat models. TERPY reduced endothelial nitric oxide synthase expression and increased reactive oxygen species production in Wistar rat aortas, but did not alter these in spontaneously hypertensive rats. Furthermore, the nitric oxide released by TERPY reacts with reactive oxygen species, decreasing their bioavailability in spontaneously hypertensive rats.

1. Introduction

Endothelial dysfunction is commonly associated with vascular diseases such as hypertension (Ghiadoni et al., 2012; McIntyre et al., 1999). It is characterized by an imbalance in the actions of the endothelium, which produces or releases less vasodilator agents than substances with vasoconstrictor activity (Rees et al., 1989). Nitric oxide, an endothelium-derived relaxing factor, is endogenously produced by the enzyme nitric oxide synthase in the presence of co-factors (Bauer and Sotníková, 2010). However, when nitric oxide synthase is uncoupled by deficiency of co-factors, it contributes to increased superoxide and decreased nitric oxide production (Roe and Ren, 2012).

Metal complexes of ruthenium release nitric oxide in a controlled way, according to the structure and the particular characteristics of each compound (Pereira et al., 2011; Lunardi et al., 2009). The nitrosyl ruthenium complex [Ru(terpy)(bdq)NO]$^{3+}$ (TERPY) is less potent, but as effective as sodium nitroprusside (SNP) (Bonaventura et al., 2007). Recently, we demonstrated that in contrast to the hypotensive effect of SNP, that of TERPY is slow, long lasting, and does not lead to reflex tachycardia (Munhoz et al., 2012).

The hypotensive effects of new nitric oxide donors were higher in hypertensive as compared with normotensive rats (de Barros et al., 2002; de Gaitani et al., 2009; Rodrigues et al., 2012; Munhoz et al., 2012). However, vasodilation induced by these agents differs

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in the experimental models of hypertension. In renal hypertensive rats, TERPY-induced vasodilation is impaired when compared with 2-kidney sham-operated rats (Rodrigues et al., 2007, 2008; Bonaventura et al., 2011). However, in spontaneously hypertensive rats it is not altered as compared with normotensive Wistar rats (Munhoz et al., 2012).

Recent findings have shown that endothelial nitric oxide synthase activity can modulate vasodilation induced by nitric oxide donors such as SNP or TERPY (Bonaventura et al., 2008, 2009). The results of Bonaventura et al. (2008) provide strong evidence that SNP induces the production of nitric oxide by endothelial cells in normotensive rat aortas through nitric oxide synthase activation. On the other hand, the relaxation induced by TERPY is impaired in aortic rings with intact endothelium of normotensive Wistar rats due to nitric oxide synthase uncoupling induced by TERPY (Bonaventura et al., 2009). When uncoupled, nitric oxide synthase leads to the production of superoxide anion and/or hydrogen peroxide (Kietadisorn et al., 2012; Montezano and Touyz, 2012).

We hypothesized that endothelial dysfunction in spontaneously hypertensive rats would lead to a different modulation of the actions of TERPY. To test this hypothesis, we evaluated the hypotensive effect and aortic relaxation induced by TERPY in the absence and presence of the nitric oxide synthase inhibitor L-N^G-Nitroarginine methyl ester (L-NAME) in spontaneously hypertensive rats and compared with normotensive Wistar rats. To verify the changes in the levels of oxidative stress and nitric oxide bioavailability after the administration of TERPY, flow cytometry analysis was performed on endothelial cells loaded with fluorescent dyes (4,5-diaminofluorescein diacetate and dihydroethidium). We also analyzed the expression of endothelial nitric oxide synthase in aortas before and after stimulation with TERPY using western blotting.

2. Materials and methods

The Animal Research Ethics Committee (CEEA) at the School of Dentistry of Araçatuba – UNESP approved all experiments conducted in this study (process number 001619-2010).

2.1. Animals

Male spontaneously hypertensive rats, 120 days of age, and with systolic blood pressure $\geq$ 150 mmHg were used in this study. Male normotensive Wistar rats served as controls. Animals were housed under standard laboratory conditions (12-h light/dark cycles at 21 °C with free access to food and water). Systolic blood pressure was evaluated by tail plethysmography (PowerLab, ADInstruments, Melbourne, Australia).

2.2. Cannula implantation surgery

The animals were anesthetized with ketamine/xylazine (45 mg/kg and 5 mg/kg, respectively) and a polyethylene cannula (PE_{10} connected to PE_{50}) filled with heparinized saline (0.15 mol/L) was inserted into the abdominal aorta, through the femoral artery, for blood pressure recording. A second cannula was inserted into the femoral vein for drug administration. The cannulas were exteriorized in the nucha, and the animals were housed separately for 24 h before the start of the experiments.

2.3. Mean arterial pressure measurement

A pressure transducer and an amplifier (AD Instruments, Melbourne, Australia) were connected to the intra-arterial cannula and blood pressure was continuously recorded in conscious and freely moving rats using a data acquisition system. The mean arterial pressure and heart rate were calculated (LabChart 4 software, ADInstruments, Melbourne, Australia) at basal conditions and during drug administration.

2.4. Evaluation of the hypotensive effects of the administered drugs

Drugs were diluted in sodium chloride 0.15 mol/L and kept unexposed to light at −20 °C. Before drug administration, the baseline mean arterial pressure and heart rate of the animals were recorded during a 30-min stabilization period. Saline (0.15 mol/L) or L-NAME (10 mg/kg) was administered intravenously. After stabilization was reached by L-NAME, 5 mg/kg TERPY was administered intravenously to the same animal. The dose of TERPY was chosen based on previous studies showing that at this dose, mean arterial pressure is reduced without harmful side effects (Munhoz et al., 2012). The variation of mean arterial pressure was calculated as the difference between the mean arterial pressure at basal condition and at the maximum pharmacological hypotensive effect after drug administration.

2.5. Determination of oxidative damage

In another set of experiments, TERPY (5 mg/kg) or saline (0.15 mol/L) was administered intravenously in Wistar rats and spontaneously hypertensive rats. After drug administration, the animals were kept undisturbed for 45 min and subsequently killed by decapitation. The blood of each animal was separately collected into tubes containing heparin (Hemofolol, 0.2 ml of a 5000 UI/ml stock solution, Cristália, Brazil) and centrifuged at 1000 rpm for 15 min at 4 °C (Centrifuge 5810R, rotor S-4-104, Eppendorf, Germany). Plasma was removed by aspiration, and erythrocytes were washed thrice with cold NaCl (0.15 mol/L). Hemolysates were prepared by the addition of $MgSO_4$ (4 mM) and acetic acid (1 mmol/L). Substances reacting to 2-thiobarbituric acid were determined in erythrocytes as an indicator of lipid peroxidation levels. Briefly, hemolysates were mixed with 1 ml of trichloroacetic acid (10%) to precipitate proteins followed by centrifugation (1000 rpm/3 min) and the subsequent addition of 1 ml of 2-thiobarbituric acid (0.67%) (Buege and Aust, 1978). The samples were heated in boiling water for 15 min. The amount of 2-thiobarbituric was determined by measuring the absorbance at 535 nm in a spectrophotometer (Hitachi U-1100, Tokyo, Japan). The results were expressed in nmol/mg protein using the appropriate molar extinction coefficient ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$). The protein concentration was measured using the method of Lowry et al. (1951) with bovine serum albumin used as standard.

2.6. Evaluation of the vascular effects of the administered drugs

After killing the rats by decapitation, the thoracic aortas were quickly removed from the animals, dissected, and cut in 4–5-mm rings. Some aortic rings were kept with intact endothelium and others had the endothelium mechanically removed. The aortic rings were placed between two stainless steel stirrups and connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain). The rings were kept in a chamber containing Krebs solution with the following composition (in mM): NaCl 130.0; KCl 4.7; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 14.9; $C_6H_{12}O_6$ 5.5; $CaCl_2$ 1.6, pH 7.4 and gassed with 95% O_2 and 5% CO_2 at 37 °C. Each ring was stretched to a resting tension of 1.5 g and kept in this state for 60 min to reach stabilization. The rings were washed regularly every 15 min. After washing and stabilizing, the rings were stimulated with phenylephrine (0.1 μmol/L) and the presence of endothelium was verified by the degree of