

A new vasodilator does not induce tolerance in rat aorta and cava vein.

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Introduction: The nitrite anion (NO_2^-) can be the major source of intravascular and tissue storage of nitric oxide (NO), important modulator of vascular tone and blood pressure control. The compound $[\text{RU}(\text{BPY})_2(\text{PY})\text{NO}_2](\text{PF}_6)$, (RuBPY), releases NO inside the vascular smooth muscle cell in a tissue dependent manner. Long-term treatment with the patients with the NO donors such as nitroglycerin, leads to the development of tolerance characterized by the rapid loss of vasodilator effects. It is believed that the tolerance is a multifactorial process and it involves increased production of vascular reactive oxygen species (ROS), decreased activity of soluble guanylyl-cyclase (sGC) and increased expression and activity of phosphodiesterases. The tolerance may be due to endothelial dysfunction. Therefore, we have hypothesized that RuBPY would induce tolerance in veins and in intact endothelium rat aorta. **Objective:** The aim of the present study was to investigate whether exposure of rat denuded or intact endothelium aortic rings or in veins for 5 minutes with RuBPY (EC_{100} : 10 $\mu\text{mol/L}$) induces tolerance to this NO donor. **Methods:** The RuBPY complex was synthesized by our research group. Male Wistar rats (200-250g) were killed under anesthesia and the inferior cava vein and thoracic aorta were quickly cut into rings of 4mm in length that were placed between two stainless-steel stirrups and connected to an isometric force transducer to measure the tension. The vessels were placed in an organ chamber containing Krebs solution maintained at pH7.4 and gassed with carbogen at 37°C. Veins were pre-contracted with endothelin-1 (ET-1, EC_{50} : 20 nmol/L) and endothelium-intact and endothelium-denuded aorta with phenylephrine (PE, EC_{50} : 100 $\mu\text{mol/L}$). After reaching a stable and maintained contraction, RuBPY (3nmol/L-5 $\mu\text{mol/L}$) was added cumulatively to the organ bath. Experiments were conducted after 5 minutes incubation (tolerance) or in the absence (control) of RuBPY followed by 20 minutes of washing. We used nitroglycerin (GTN), a classical NO donor, as a positive control of tolerance. The parameters of maximum effect (E_{max}) and Potency (pD_2) were analyzed. Results are expressed as mean \pm SEM. Statistical significance was determined by using the Student's *t* test. In all cases, probability levels of less than 0.05 ($P < 0.05$) were taken to indicate statistical significance. All pharmacological studies were performed in accordance with the Ethical Animal Committee of the University of São Paulo (2012.1.134.53.12). **Results:** The compound RuBPY induced concentration-dependent relaxation in veins (pD_2 : 7.21 \pm 0.33; E_{max} : 92.8 \pm 3.4%, $n=7$, $P < 0.05$) and aortas with endothelium (pD_2 : 8.21 \pm 0.41; E_{max} : 101.1 \pm 1.1%, $n=3$, $P < 0.05$) and without endothelium (pD_2 : 7.39 \pm 0.05; E_{max} : 100.83 \pm 1.77%, $n=3$, $P < 0.05$). It was observed that the incubation with RuBPY, for 5 minutes followed by 20 minutes of washing did not affect the E_{max} induced by the compound in veins (pD_2 : 7.25 \pm 0.37; E_{max} : 80.2 \pm 2.5%, $n=7$, $P < 0.05$) neither intact endothelium aorta (pD_2 : 7.92 \pm 0.27; E_{max} : 101.1 \pm 1.1%, $n=3$, $P < 0.05$). Similarly, no changes on the parameters studied were observed in denuded arteries incubated with RuBPY (pD_2 : 7.34 \pm 0.25, E_{max} : 99.4 \pm 4.5%, $n=4$, $P < 0.05$). The incubation with GTN (EC_{100}) for 5 minutes followed by 20 minutes of washing reduced the E_{max} in veins (for: 75.35 \pm 2.8%, to: 53.22 \pm 1.2% $n=6$, $P < 0.05$). **Conclusion:** Our data demonstrate that RuBPY does not induce tolerance in rat aorta and inferior cava vein and GTN induced tolerance in inferior cava vein, after incubation for 5 minutes.

Keywords: NO donor, rat aorta, rat vein, tolerance.

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