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A new vasodilator does not induce tolerance in rat aorta.

TM Banin, RS Da Silva, LM Bendhack. *Faculty of Pharmaceutical Sciences - University of São Paulo, Physics and Chemistry, Brazil*

Introduction: The nitrite anion (NO_2^-) can be the major source of intravascular and tissue storage of nitric oxide (NO), an important modulator of vascular tone and blood pressure control. The compound $[RU(BPY)_2(PY)NO_2](PF_6)]$, (RUBPY), releases NO inside the vascular smooth muscle cell in a tissue dependent manner. Long-term treatment with the patients with the clinical use of 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 process is a multifactorial process and 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 intact endothelium rat aorta. Aim: The aim of the present study was to investigate whether exposure of rat aortic rings with or without endothelium, for 5 minutes with RuBPY (EC_{100}:10µmol/L) induces tolerance to this NO donor. Methods: The RuBPY complex was synthesized as previously reported and characterized by UV-Visible spectroscopy as well as cyclic voltammetry experiments. Male Wistar rats (200-250g) were killed under anesthesia and the thoracic aorta was 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 rings were placed in an organ chamber containing Krebs solution maintained at pH 7.4 and gassed with carbogen, at 37℃. Endothelium-intact and endothelium-denuded tissues were pre-contracted with phenylephrine (EC_{50}: 100µmol/L), prepared in deionized water. After reaching a stable and maintained contraction, RuBPY (3nmol/L–5µmol/L), prepared in pH 7.4 phosphate buffer (1mmol/L) was added cumulatively to the organ bath. Experiments were conducted after 5 min incubation (tolerance) or in the absence (control) of RuBPY (10µmol/L). The parameters of maximum effect (Emax) and Potency (pD_2) were analyzed. Results are expressed as mean ± 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 aortas with endothelium (pD_2:7.81 ± 0.18; Emax: 101.6 ± 1.4%, n=7, $P<0.05$) and without endothelium (pD_2:7.54 ± 0.13; Emax: 103.4 ± 0.4%, n=6, $P<0.05$). It was observed that the incubation with RuBPY EC_{100}, for 5 minutes followed by 1 hour of washing did not affect the maximum relaxation induced by the compound in intact endothelium aorta (pD_2:7.99 ± 0.18; Emax: 98.5 ± 1.6%, n=5, $P<0.05$). Similarly, no changes on the parameters studied were observed in denuded arteries incubated with RuBPY (pD_2:7.89 ± 0.18, Emax: 100.0 ± 0.4%, n=6, $P<0.05$). Conclusion: Our data demonstrate that RuBPY (EC_{100}) does not induce tolerance in rat aorta after incubation for 5 minutes. Financial Support: CNPq and FAPESP.