Vascular Pharmacology 63 (2014) 55–62

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Vasorelaxant effects of 1-nitro-2-phenylethene in rat isolated aortic rings

Loeste Arruda-Barbosa [a], Karilane Maria Silvino Rodrigues [a], Francisco das Chagas Vasconcelos Souza-Neto [a], Gloria Pinto Duarte [b], Rosivaldo S. Borges [c], Pedro Jorge Caldas Magalhães [a], Saad Lahlou [a,*]

[a] Department of Physiology and Pharmacology, Federal University of Ceará, CE, Brazil
[b] Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife, PE, Brazil
[c] Department of Pharmacy, Federal University of Pará, Belém, PA, Brazil

ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form 27 July 2014
Accepted 1 August 2014
Available online 15 August 2014

Chemical compounds studied in this article:
1-Nitro-2-phenylethene
(PubChem CID: 5284459)

Keywords:
Isolated thoracic aorta
Endothelium-independent vasorelaxation
Myogenic
1-Nitro-2-phenylethene
Normotensive rat

ABSTRACT

Previously, we showed that nitro-2-phenylethane is a vasorelaxant constituent of the essential oil of *Aniba canelilla*. Here, we investigated the mechanisms underlying the vascular effects of 1-nitro-2-phenylethene (NPe), a structural analog of 1-nitro-2-phenylethane obtained synthetically, in rat isolated thoracic aortic preparations. At 0.1–100 μg/mL, NPe similarly relaxed endothelium-intact or endothelium-denuded aortic preparations pre-contracted with 60 mM KCl or with phenylephrine (PHE, 1 μM). Vasorelaxant effects of NPe against PHE-induced contractions remained unaffected following blockade of potassium channels by TEA, and inhibition of either nitric oxide synthase by L-NAME, cyclooxygenase by indomethacin or guanylate cyclase by ODQ. In preparations maintained under Ca^{2+}-free conditions, NPe significantly reduced the contractions induced (i) by PHE, but not those evoked by caffeine, (ii) by $CaCl_2$ in either PHE (in the presence of 1 μM verapamil)- or KCl-stimulated preparations, (iii) by extracellular Ca^{2+} restoration in thapsigargin-treated aortic preparations, and (iv) by the activator of protein kinase C phorbol-12,13-dibutyrate or the inhibitor of protein tyrosine phosphatase sodium orthovanadate. It is concluded that NPe induced an endothelium-independent vasorelaxation with potency greater than its structural analog 1-nitro-2-phenylethane. Such action appears to occur intracellularly probably through inhibition of contractile events that are clearly independent of Ca^{2+} influx from the extracellular milieu.

1. Introduction

Hypertension is a common and progressive disorder which constitutes a major risk for diabetes, stroke, as well as cardiovascular and renal diseases. A great number of researches focused on the bioactive compounds from natural resources as potential substances for the treatment of hypertension. Efforts to this effect have been pursued by our group for a number of years to identify novel antihypertensive compounds with a vasodilator activity especially derived from essential oils of aromatic plants from the north–northeast region of Brazil [1–8]. Nitroderivatives found in higher plants are rare. 1-Nitro-2-phenylethane is the first nitro compound isolated from plants [9], and is considered to be responsible for the plant's cinnamon scent [10]. It is the main constituent of the essential oil of *Aniba canelilla* (H.B.K.) Mez [syn. *Aniba elliptica* A. C. Sm., *Cryptocarya canelilla* Kunth] (EOAC), (Lauraceae), an aromatic plant abundant in the Amazon region commonly known as "casca-preciosa" (precious bark).

Previously, we showed that intravenous (i.v.) administration of 1-nitro-2-phenylethane (Fig. 1A) induced two periods of hypotension and bradycardia in either normotensive [11] or hypertensive [12] rats. Initially a rapid bradycardia (onset time of 1–2 s) occurred coincidentally (onset time of 2 s) with an arterial hypotension (phase 1) and then, a delayed decrease in blood pressure (peak between 4 and 10 s) associated with a second bradycardia (phase 2) [11,12]. Several lines of evidence confirmed that phase 1 (rapid bradycardiac and depressor responses to EOAC and 1-nitro-2-phenylethane) was of reflex origin (vago-vagal reflex). Phase 1 appears not to involve the activation of either vanilloid $TPRV_1$ or 5-HT_3 receptors located on vagal sensory nerves [11,12]. The second hypotensive response (phase 2) of 1-nitro-2-phenylethane results from a direct vasodilatory effect, as evidenced by the concentration-dependent reduction of phenylephrine (PHE)-

Abbreviations: cGMP, cyclic guanosine-5′-monophosphate; EGTA, ethylene glycol tetraacetic acid; EOAC, essential oil of *Aniba canelilla*; i.v., intravenous; IP_3, inositol triphosphate; KHS, Krebs–Henseleit solution; L-NAME, L-N(G) nitroarginine methyl ester; NO, nitric oxide; NPe, 1-nitro-2-phenylethene; ODQ, 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one; PDB, phorbol-12,13-dibutyrate; PHE, phenylephrine; ROCCs, receptor-operated channels; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+} pump; sGC, soluble guanylate cyclase; SOCCs, store-operated Ca^{2+} channels; TEA, tetraethylammonium chloride; VOCCs, voltage-operated calcium channels.

* Corresponding author at: Department of Physiology and Pharmacology, Federal University of Ceará, Rua Cel. Nunes de Melo 1127, Fortaleza, CE, Brazil. Tel.: +55 85 33668334; fax: +55 85 33668333.
E-mail addresses: loeste.arruda@gmail.com (L. Arruda-Barbosa), karilane.fisio@gmail.com (K.M.S. Rodrigues), vasconcelos.18@gmail.com (F.C.V. Souza-Neto), duarte.gloria@gmail.com (G.P. Duarte), lqfmed@gmail.com (R.S. Borges), pjcmagal@ufc.br (P.J.C. Magalhães), lahlou562@gmail.com (S. Lahlou).

http://dx.doi.org/10.1016/j.vph.2014.08.002
1537-1891/

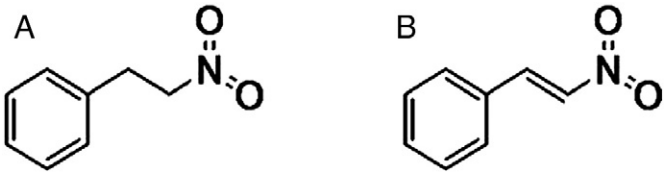

Fig. 1. Chemical structure of 1-nitro-2-phenylethane (A) and its structural analog 1-nitro-2-phenylethene (B).

induced contractions in either isolated aortic [11] or superior mesenteric artery [12,13] preparations. Recently, it was shown that the vasodilator actions of 1-nitro-2-phenylethane appeared to derive from its ability to augment cGMP levels in aortic smooth muscle cells, a finding suggestive for the involvement of stimulating the soluble guanylate cyclase (sGC)–cGMP pathway in such vasodilator effects [14].

The sp^3 carbon atoms are responsible for different conformations of 1-nitro-2-phenylethane. Thus, a conformational restriction by substitution of the alkane for the alkene moiety allows the formation of 1-nitro-2-phenylethene (NPe, Fig. 1B) which may exhibit different degrees of potency or different types of activities. Therefore, the present investigation was undertaken to assess the vascular effects of NPe in rat isolated thoracic aortic preparations and the putative mechanisms underlying these effects.

2. Materials and methods

2.1. Synthesis of 1-nitro-2-phenylethene

NPe or 1-((E)-2-nitro-vinyl)-benzene (β-nitrostyrene) was synthesized by employing the Claisen–Schmitd's procedure [15,16] using benzaldehyde and nitromethane as substrates (0.1 and 0.12 eq., respectively). The benzaldehyde was 'one-pot' converted, in 89% yield, to the corresponding styrene by treatment with 0.05 eq. of NaOH in methanol and water at 0–10 °C.

The resulting precipitate was filtered out and dried under vacuum to give the desired styrene derivative, NPe. The *trans* product is more preferential than *cis* due to steroselective reactions. The title compound was crystallized in ethanol as a cream-colored solid; m.p. 56.2–58.6 °C (55–58 °C; Sigma-Aldrich standard). IR ν_{max} 1600, 1550, 1498, 1375 cm^{-1}; ^1H NMR (CD_3SOCD_3, 200 MHz) δ 7.2–7.3 (d, 2 H), 7.35–7.4 (d, 2H), 7.55 (d, 1H), 7.7 (d, 1H), 7.9 (d, 1H); ^{13}C NMR (CD_3SOCD_3, 50 MHz) δ 127.52, 128.84, 129.69, 136.82, 137.99, 140.96. The final product was identified by NMR (^1H NMR) and FT-IR spectroscopic techniques and compared with literature [17].

2.2. Animals

Adult male Wistar rats (280–340 g) were kept under conditions of constant temperature (22 ± 2 °C) with a 12 h light/12 h dark cycle and free access to food and water. All animals were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996). All procedures described here were reviewed by and had prior approval from local animal ethics committee (11043633-4).

2.3. Tissue preparation and experimental protocols

Rats were sacrificed by asphyxia induced by CO_2 inhalation. For isometric tension recording, thoracic aorta was removed and placed in cold oxygenated KHS buffer. Segments of this artery (3 mm in length), free of fat and connective tissue, were mounted between two steel hooks in a 5 mL isolated tissue chamber containing gassed (95% O_2 and 5% CO_2) Krebs-Henseleit solution (KHS), at 37 °C, under a resting tension of 1 g, which was readjusted every 15 min during a 45-min equilibration period before drug administration. Isometric tension was recorded by using an isometric force–displacement transducer (Grass Model FTO3, Quincy, MA, USA) connected to an acquisition system (PM-1000; CWE Inc., Akron, OH, USA). Vessels were initially exposed twice to 60 mM KCl to check their functional integrity. After 30 min, rings were contracted with a concentration (0.1 μM) of PHE inducing 50–70% of the contraction induced by KCl and acetylcholine (1 μM) was then added to assess endothelium integrity. Sixty min later, the series of experiments 1 to 8 were performed.

2.3.1. Series 1

This series of experiments was carried out to assess the effects of NPe (0.1–100 μg/mL) on the resting tone of endothelium-containing aortic preparations. In other experiments, effects of vehicle at the same concentrations used to dissolve NPe were also studied.

2.3.2. Series 2

In this series of experiments, the effects of cumulative concentrations (0.1–100 μg/mL) of NPe on the sustained contractile responses to KCl (60 mM) or PHE (1 μM) were studied in either endothelium-intact or endothelium-denuded aortic ring preparations maintained in Ca^{2+}-containing medium. In order to assess the role of the sGC, nitric oxide (NO) synthase, prostaglandins or potassium channels in the effects of NPe in PHE-contracted tissues, experiments were performed in endothelium-containing aortic ring preparations incubated for 20 min with ODQ (10 μM), L-NAME (100 μM), indomethacin (10 μM) or TEA (5 mM), respectively. Effects of vehicle at the same concentrations used to dissolve NPe were also studied.

2.3.3. Series 3

This series of experiments was carried out to assess the inhibitory effects of NPe on the contractions induced by exogenous addition of Ca^{2+} in aortic ring preparations depolarized by KCl (60 mM) in a Ca^{2+}-free medium. Calcium-free solutions were prepared by omitting $CaCl_2$ from normal KHS. After verification of tissue responsiveness in a Ca^{2+}-containing medium, the preparation was maintained in the Ca^{2+}-free KHS in the presence of 60 mM K^+ and EGTA (50 μM) to promote voltage-operated calcium channel (VOCC) activation. Thereafter, a cumulative concentration–response curve for Ca^{2+} (0.1–20 mM) was then performed. After a washing preparation by changing the bath chamber solution to remove just Ca^{2+} from the medium, NPe (at 1 or 3 μg/mL) was added to the preparation for 5 min, and their effects on cumulative concentration–response curve for $CaCl_2$ (0.1–20 mM) were then evaluated. Maximal response to exogenous Ca^{2+} was obtained when an increase in the Ca^{2+} concentration did not induce a significant additional contraction. The contractile response obtained with the first concentration–response curve for $CaCl_2$ was taken as control, and the contractions were calculated as a function of the value observed to 20 mM Ca^{2+}.

2.3.4. Series 4

The same protocol as that of series 3 (construction of cumulative concentration–response curve for Ca^{2+} from 0.1 to 20 mM) was performed in aortic ring preparations under Ca^{2+}-free conditions. These preparations were stimulated with PHE (1 μM) in the presence of verapamil (1 μM) to obtain contractile responses preferentially mediated by receptor-operated calcium channels (ROCCs).

2.3.5. Series 5

To investigate whether NPe inhibitory actions could also be effective on contractions elicited by sarcoplasmic reticulum Ca^{2+} channels activated by inositol triphosphate (IP_3), the effects of NPe on PHE-induced contractions in Ca^{2+}-free medium were determined as follows. After the usual stabilization time, the tissues were washed with Ca^{2+}-free solution for 6 min (with 1 mM EGTA). PHE (1 μM) was added to produce a transient contraction. After washing the tissues with Ca^{2+}-containing