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Endothelium-independent vasodilator effect of 2-nitro-1-phenyl-1-propanol on mesenteric resistance vessels in rats

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ABSTRACT

2-Nitro-1-phenyl-1-propanol (NPP) is a nitro alcohol with vasodilator activity in the rat aorta. The present study investigated the vasodilator properties of NPP in small vessels of the mesenteric bed, which, contrary to the aorta, contains resistance vessels. Using myography, isometric contractions were recorded in rings of second- and third-order branches from the rat mesenteric artery. NPP relaxed mesenteric ring preparations that were contracted with phenylephrine, U-46619, and KCl (40 mM), resulting in significantly different EC_{50} values (0.41 μM [0.31–0.55 μM], 0.16 μM [0.10–0.24 μM], and 4.50 μM [1.86–10.81 μM], respectively). NPP-induced vasodilation decreased as the extracellular K^+ concentration increased. The pharmacological blockade of K^+ channels with tetraethylammonium, $BaCl_2$, CsCl, and apamin also blunted NPP-induced vasorelaxation. In contrast, NPP-induced vasodilation was resistant to indomethacin, L-N^G-nitroarginine methyl ester (L-NAME), and endothelium removal, indicating that neither prostaglandins nor the endothelial release of nitric oxide is involved in the relaxant effects of NPP. Conversely, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), cis-*N*-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine hydrochloride (MDL-12,330 A), and H-89 reduced NPP-induced vasodilation. Under Ca^{2+}-free conditions, NPP did not alter transient contractions that were evoked by caffeine, but it reduced transient contractions that were evoked by phenylephrine. In mesenteric rings that were loaded with the fluorescent Ca^{2+} indicator Fluo-4 AM and stimulated with phenylephrine, NPP blunted both contractions and fluorescence signals that were related to cytosolic Ca^{2+} levels. In conclusion, the vasodilatory actions NPP on mesenteric vessel resistance involved the participation of cyclic nucleotides and the opening of K^+ channels.

1. Introduction

The nitro alcohol 2-nitro-1-phenyl-1-propanol (NPP; CAS no. 6343-57-3) is structurally close to norephedrine, a sympathomimetic agonist that is useful as an upper respiratory decongestant (Bravo, 1988; Meltzer et al., 2010). Chemically, they differ from one another because NPP possesses a NO_2 functional group, whereas norephedrine contains a NH_2 group. Such substituents likely determine the pharmacological profile of these compounds. Despite the well-known vasoconstrictor properties of norephedrine (Persson et al., 1973), NPP has been shown to exert vasodilator effects in isolated rat aorta (Brito et al., 2016), but it unlikely acts as an adrenergic ligand.

As reported in aortic smooth muscle (Brito et al., 2016), the induction of vasodilation by NPP appears to occur through the stimulation of guanylate cyclase, which augments the intracellular levels of cyclic guanosine monophosphate (cGMP). Cellular responses to cGMP involve target proteins, known as cGMP-dependent protein kinases (e.g., protein kinase G [PKG]; Lucas et al., 2000). Once activated, targeted proteins may induce other events that result in lower Ca^{2+} release from intracellular stores (Xia et al., 2001) or higher Ca^{2+} sequestration to the sarcoplasmic reticulum (Koller et al., 2003). Vasorelaxant compounds that stimulate guanylate cyclase can also modulate the gating of K^+ channels, resulting in pronounced hyperpolarization and the inhibition of force in vascular smooth muscle (Kubo et al., 1994). In the rat mesenteric artery, several types of K^+ channels appear to mediate vasorelaxant actions that are induced by the release of endothelium-derived hyperpolarizing factor (EDHF; Hilgers et al., 2006).

Endothelium-derived mechanisms that are involved in muscle tone regulation in small vessels differ from conduit vasculature in several aspects. For example, nitric oxide (NO) is the predominant factor in large conduit vessels, whereas EDHF appears to contribute more

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significantly as the vessel size decreases (Clark and Fuchs, 1997; Hilgers et al., 2006; Shimokawa et al., 1996). The vasodilator effects of NPP on the rat aorta suffered no change under blockade of K^+ channels with tetraethylammonium, despite the higher levels of cGMP. Additionally, its relaxant effects decreased weakly in the presence of an adenylate cyclase inhibitor (Brito et al., 2016). In the present study, we evaluated whether NPP interferes with cellular events that are related to the guanylate cyclase-K^+ channel pathway to produce vasodilator effects in small arteries of the rat mesenteric bed.

2. Materials and methods

2.1. Animals

Male Wistar rats (220–280 g) were obtained from the institutional facilities of the Department of Physiology and Pharmacology, Federal University of Ceará, Fortaleza, Brazil. The rats were housed under standard conditions with free access to food and water. All of the animals were handled in accordance with the Ethical Principles for the Care and Use of Laboratory Animals, published by the Brazilian National Council for Animal Experimentation (RN12/2013), and with approval from the institutional animal Ethics Committee (no. 22/2014).

2.2. Solutions and drugs

We used modified Krebs-Henseleit solution (MKHS; 118.0 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4{\cdot}7H_2O$, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, and 11.0 mM glucose) as the physiological medium to maintain the viability of isolated tissues. Salts that were used to prepare MKHS were obtained from Merck (Darmstadt, Germany) and Vetec (Rio de Janeiro, Brazil). For the experiments under Ca^{2+}-free conditions, $CaCl_2$ was omitted from the normal MKHS, and ethylene glycol-bis(*b*-aminoethyl ether)*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA; 1 mM) was added. NPP was purchased from Sigma (St. Louis, MO, USA), dissolved directly in MKHS at 2 mM, and sonicated just before use. To induce sustained contractions in isolated preparations of mesenteric vessels, we used either phenylephrine, depolarizing MKHS that contained high KCl concentrations, or the thromboxane A_2 analogue U-46619 as a contractile stimulus. Some of the experiments with NPP were conducted with vascular preparations that were pretreated (10–20 min before applying the contractile stimulus) with the K^+ channel inhibitors tetraethylammonium, glibenclamide, 4-aminopyridine (4-AP), $BaCl_2$, CsCl, and apamin, the nitric oxide synthase inhibitor L-NG-nitroarginine methyl ester (L-NAME), the cyclooxygenase inhibitor indomethacin, the adenylate cyclase inhibitor cis-*N*-(2-phenylcyclopentyl)-azacyclotridec-1-en-2-amine hydrochloride (MDL-12,330 A), the guanylate cyclase inhibitor 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), or the protein kinase A inhibitor *N*-[2-(*p*-bromocinnamylamino)ethyl]–5-isoquinolinesulfonamide dihydrochloride (H-89). All of the reagents were obtained from Sigma (St. Louis, MO, USA). The Ca^{2+} fluorophore Fluo-4/AM was purchased from Life Technologies (Carlsbad, CA, USA).

2.3. Contractility of isolated mesenteric vessels

The rats were euthanized under 2,2,2-tribromoethanol anesthesia (250 mg/kg, i.p.). The mesenteric vascular bed was removed and placed in MKHS at room temperature. Second- or third-order branches of the mesenteric artery (approximately 2 mm long segments) were mounted in a 610M-DMT Wire Myograph System (Danish Myo Technology, Aarhus, Denmark) by two tungsten wires (outer diameter 40 μm; approximately 2 cm length). The wires passed through the lumen of each ring, one fixed to a micrometer for length adjustments and the other connected to a force transducer according to the method described by Mulvany and Halpern (1977). The organ bath contained warmed (37 °C) MKHS that was continuously bubbled with 5% CO_2 in O_2. After a 30 min equilibration period, the segments were stretched to their optimal lumen diameter for active tension development. The optimal lumen diameter was determined based on the internal circumference/wall tension ratio for each segment by setting the internal circumference, L_0, to 90% of what the vessels would have if they were exposed to a passive tension that is equivalent to the tension produced by transmural pressure of 100 mmHg (Mulvany and Halpern, 1977). This procedure was performed using specific software for the normalization of resistance arteries (DMT Normalization Module, AD Instruments, Bella Vista, Australia). For endothelium denudation, a human hair was inserted into the lumen of the arteries, and friction movements were made against the arterial wall. The lack of endothelial functionality was pharmacologically confirmed by the absence of relaxation after the addition of acetylcholine (1 μM) on the plateau of a K^+(60 mM)-induced contraction.

2.4. Simultaneous measurement of tension and intracellular Ca^{2+} concentration in rings of mesenteric vessels

Vessel segments from the second-order branch of the superior mesenteric artery were dissected and maintained in oxygenated MKHS at room temperature. After removing adherent fat under a microscope, cylindrical ring-like segments were obtained and carefully mounted as previously described for the experiments that used the Wire Myograph System. To measure the levels of intracellular Ca^{2+}, a confocal myograph (DMT120CW Confocal Wire Myograph, Aarhus, Denmark) allowed the capturing of high-resolution images using fluorescent markers.

Under constant temperature (37 °C), a resting tension of 11.5 kPa was applied to each isolated artery segment. The preparation was incubated for 30 min in the absence of light with the fluorescent Ca^{2+} indicator Fluo-4 AM (5 μM) that was supplemented with pluronic acid (0.1%, v/v; Life Technologies, Carlsbad, CA, USA). After washing to remove the extracellular dye, Ca^{2+} fluorescence was detected with an inverted confocal microscope (Olympus, IX81) at 20× magnification and excitation/emission wavelengths of 488/505–515 nm. The sampling rate was 1 frame/2.5 s. Intracellular Ca^{2+} variations are expressed as the ratio F/F_0, where F is the intensity of fluorescence that was recorded during the course of the experiment, and F_0 is the intensity of fluorescence at the start of the experiment. Simultaneously, variations in tension were recorded with an isometric force transducer that was connected to a data acquisition system (PowerLab 8/30, AD Instruments).

2.5. Statistical analysis

The data are expressed as mean ± the standard error of the mean (S.E.M.), and *n* indicates the number of experiments. The EC_{50} (i.e., the concentration of NPP at which 50% of a contractile response was inhibited) values were calculated by interpolation from semi-logarithmic plots and are expressed as geometric means [95% confidence interval]. Statistical significance ($P < 0.05$) was assessed using the Mann-Whitney *U*-test and one- or two-way analysis of variance (ANOVA), followed by the Holm-Sidak *post hoc* test when appropriate.

3. Results

3.1. Vasorelaxant effects of NPP on rat isolated mesenteric vessels

Endothelium-intact mesenteric artery rings that were incubated in Ca^{2+}-containing medium were initially precontracted with either 10 μM phenylephrine (n=7; corresponding to 16.0 ± 1.8 mN) or 1 μM U-46619 (n==7; 11.8 ± 1.4 mN). NPP (0.01–10 μM) was added during the steady-state contraction and fully relaxed the preparations in a concentration-dependent manner ($P < 0.001$, one-way ANOVA;