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Niclosamide ethanolamine inhibits artery constriction

Shan-Liang Li[a], Jie Yan[a], Yan-Qiu Zhang[a], Chang-Lin Zhen[a], Ming-Yu Liu[a], Jing Jin[a], Jin-Lai Gao[a], Xiao-Lin Xiao[a], Xin Shen[a], Yu Tai[a], Nan Hu[a], Xin-Zi Zhang[a], Zhi-Jie Sun[b], De-Li Dong[a,*]

[a] Department of Pharmacology (The State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Translational Medicine Research and Cooperation Center of Northern China, Heilongjiang Academy of Medical Sciences, Harbin Medical University, PR China
[b] Institute of Materials Processing and Intelligent Manufacturing & Center for Biomedical Materials and Engineering, Harbin Engineering University, PR China

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ABSTRACT

We previously demonstrated that the typical mitochondrial uncoupler carbonyl cyanide m-chlorophenylhydrazone (CCCP) inhibited artery constriction, but CCCP was used only as a pharmacological tool. Niclosamide is an anthelmintic drug approved by FDA. Niclosamide ethanolamine (NEN) is a salt form of niclosamide and has been demonstrated to uncouple mitochondrial oxidative phosphorylation. The aim of the present study was to elucidate the vasoactivity of NEN and the potential mechanisms. Isometric tension of rat mesenteric artery and thoracic aorta was recorded by using multi-wire myograph system. The protein levels were measured by using western blot techniques. Niclosamide ethanolamine (NEN) treatment relaxed phenylephrine (PE)- and high K^+ (KPSS)-induced constriction, and pre-treatment with NEN inhibited PE- and KPSS-induced constriction of rat mesenteric arteries. In rat thoracic aorta, NEN also showed antagonism against PE- and KPSS-induced constriction. NEN induced increase of cellular ADP/ATP ratio in vascular smooth muscle cells (A10) and activated AMP-activated protein kinase (AMPK) in A10 cells and rat thoracic aorta. NEN-induced aorta relaxation was attenuated in AMPKα1 knockout (-/-) mice. SERCA inhibitors cyclopiazonic acid and thapsigargin, but not K_{ATP} channel blockers glibenclamide and 5-hydroxydecanoic acid, attenuated NEN-induced vasorelaxation in rat mesenteric arteries. NEN treatment increased cytosolic $[Ca^{2+}]_i$ and depolarized mitochondrial membrane potential in vascular smooth muscle cells (A10). Niclosamide in non-salt form showed the similar vasoactivity as NEN in rat mesenteric arteries. Niclosamide ethanolamine inhibits artery constriction, indicating that it would be promising to be developed as an anti-hypertensive drug or it would induce vasodilation-related side effects when absorbed *in vivo*.

1. Introduction

Hypertension is a major cardiovascular disease worldwide [1]. Hypertension is not an isolated event, and it is generally accompanied with other metabolic disorders, such as diabetes, hyperlipidemia, and obesity. Combined with these metabolic disorders significantly, hypertension amplifies the risk of occurrence of stroke, atherosclerosis, heart infarction, retinopathy, and nephropathy. Therefore, it is ideal to develop a type of anti-hypertensive drugs with simultaneous benefits for both diabetes and hyperlipidemia.

Abbreviations: Ach, acetylcholine chloride; AMPK, AMP-activated protein kinase; CCCP, carbonyl cyanide m-chlorophenylhydrazone; CPA, cyclopiazonic acid; GLI, glibenclamide; 5-HD, 5-hydroxydecanoic acid; mitoROS, mitochondrial reactive oxygen species; Pax, paxilline; PE, phenylephrine; PSS, physiological salt solution; TMRM, tetramethylrhodamine methyl ester; SERCA, sarco-endoplasmic reticulum Ca^{2+} ATPase; NEN, niclosamide ethanolamine.

* Corresponding author at: Department of Pharmacology, Harbin Medical University, Baojian Road 157, Harbin 150086, Heilongjiang Province, PR China.
E-mail address: dongdeli@ems.hrbmu.edu.cn (D.-L. Dong).

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Recently, the concept of mild mitochondrial uncoupling is proposed as a protective therapeutic strategy for many disorders because that it uncouples ATP synthesis from oxidative phosphorylation due to the inducible proton-leak across the mitochondrial inner membrane, thus mitigates mitochondrial reactive oxygen species (ROS) production [2]. Mitochondrial uncoupling can be induced by mitochondrial uncoupling proteins (UCPs) which are members of the mitochondrial anion carrier family, and by mitochondrial uncouplers which are small chemicals including 2,4-dinitrophenol (DNP) and carbonylcyanide-m-chlorophenylhydrazone (CCCP) et al. The proton-leak has a marked influence on the cellular energy metabolism, therefore, the roles of mild mitochondrial uncoupling in diabetes, obesity and hyperlipidemia are extensively investigated, for instance, increases of uncoupling protein-1(UCP1) and UCP3 expression are protective against obesity and type II diabetes mellitus [3].

We have systematically investigated the effects and mechanisms of chemical mitochondrial uncoupler CCCP on the vascular function. We found that CCCP inhibited vasoconstriction [4]. However, CCCP can only be used as a pharmacological tool instead of being applied in clinic. Niclosamide is an anthelmintic drug approved by FDA (USA) that uncouples the mitochondria of parasitic worms. Niclosamide ethanolamine (NEN), the salt form of niclosamide, induces mild mitochondrial uncoupling in mice liver, and prevents hepatic steatosis and insulin resistance induced by a high-fat diet in mice. Moreover, it improves glycemic control and delays disease progression in db/db mice [5]. These results enlighten us to explore the vasoactivity of NEN since that NEN has been proven to be a mitochondrial uncoupler, like CCCP which inhibits vasoconstriction [4]. Here, we found that NEN inhibited artery constriction.

2. Materials and methods

2.1. Chemicals

Phenylephrine (PE) was purchased from Shanghai Harvest Pharmaceutical Co. Ltd (Shanghai, China). Niclosamide ethanolamine (NEN) was purchased from Shanghai Rongbai biological technology Co. Ltd (Shanghai, China). Niclosamide was purchased from Jianglai Reagent Company (Shanghai, China). 5-hydroxydecanoic acid (5-HD), cyclopiazonic acid (CPA), thapsigargin, acetylcholine chloride (Ach) were purchased from Sigma Aldrich Chemistry (Saint Louis, MO, USA). MitoSOX, fluo-3/AM, TMRM and Hoechst were purchased from life technology (Invitrogen, Oregon, USA). Glibenclamide was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Paxilline was purchased from Cayman Chemical Company (Ann Arbor, Michigan, USA). AMPK(CST #2532), p-AMPK (Thr172)(CST #2535) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). PE and Ach were dissolved in distilled water, and others were dissolved in DMSO (Tianjin Fuyu Fine Chemical Co. Ltd).

2.2. Cell culture

Arterial smooth muscle cells (A10) were purchased from American Type Culture Collection. A10 cells were cultured in DMEM medium (high glucose) containing 15% FBS and 1% Penicillin/Streptomycin at 37 °C/5% CO_2. Cells were used within 8 passages.

2.3. Animals

The adult Sprague-Dawley rats (male, body weight 320–350 g, 8-10 weeks) were purchased from Charles River (Charles River Laboratory Animal, Beijing, China). The AMPK α1 knockout (-/-) mice (background C57BL/6 strain, 8 weeks) were kindly gifted by Prof. Hong-Liang Li (Collaborative Innovation Center of Model Animal Wuhan University). Total eighty rats, six wide-type mice and two AMPK α1 knockout (-/-) mice were used in the study. Rats were maintained in groups of six animals per cage (1300 cm^2) and mice in groups of three to five animals per cage (530 cm^2) on a 14 h light and 10 h dark circadian rhythm with water and food available ad libitum. The AMPK α1 knockout (-/-) mice were anesthetized by sodium pentobarbitone (50 mg/kg, ip) and then killed. Genotyping of AMPK α1 knockout (-/-) mice by PCR was performed using the following three primers as prescribed in our previous work [4]: Primer 1: 5′-GCTTAGGTCCTCATCACTG-3′, 5′-CTTGATCATCAACTCCCAGG-3′, 5′-CGCCTTCTTGACGAGTTC-3′. Their wild-type (WT) littermates were as control. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, PR China.

2.4. Rat mesenteric artery and thoracic aorta preparation

The methods in detail were described as in our previous studies [4,6,7]. Adult male Sprague-Dawley rats were sacrificed after anesthetized by sodium pentobarbitone (40 mg/kg, ip). The entire mesentery and thoracic aorta was removed quickly, then transferred into cold (4 °C) modified physiological salt solution (PSS) with the following composition (mM): NaCl, 130; KCl, 4.7; $MgSO_4 \bullet 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5 (pH7.35-7.45). Fat tissue of mesenteric artery and thoracic aorta was separated. The mesenteric arteries and thoracic aorta were dissected into 2 mm and 3–4 mm rings respectively.

2.5. Isometric tension recording of mesenteric artery and thoracic aorta

The methods in detail were described as in our previous studies [4,7]. The mesenteric artery and thoracic aorta rings were randomized for different treatments. Mesenteric arterial ring was mounted between two wires, and fixed in the bath filled with 5 mL PSS and were continuously bubbled with gas (95% O_2 + 5% CO_2). Thoracic aortic rings were mounted in triangle-shape hook and then suspended in the bath filled with 10 ml PSS and were continuously bubbled with gas (95% O_2 + 5% CO_2). The isometric contractions of mesenteric arterial rings were measured by using multi wire myograph system (model 620 DMT, Danish Myo Technology, Denmark), and the isometric contractions of thoracic aortic rings were measured by using multi-channel myograph system (BL-420S, Chengdu Taimeng Software Co. Ltd, China). The arterial rings were equilibrated for 60 min, and then a wake-up protocol was performed to reactivate the mechanical, function, and signaling properties of the vessels through using high K^+ PSS (KPSS) and phenylephrine (PE) stimuli. The KPSS (60 mM K^+) solution for inducing vasoconstriction was composed of (mM): NaCl, 74.7; KCl, 60; $MgSO_4 \bullet 7H_2O$, 1.17; KH_2PO_4, 1.18; $NaHCO_3$, 14.9; $CaCl_2$, 1.6; D-glucose, 5.5; EDTA, 0.026. In order to avoid the error induced by natural rundown of the artery tension, we calculated the relaxation ratio of NEN by subtracting the relaxation ratio of corresponding control (DMSO). Because there is natural relaxation of artery tension in control group, the analyzed relaxation ratio of NEN will look less compared with the original recording traces after subtracting the relaxation ratio of corresponding control (DMSO).

2.6. Measurement of mitochondrial reactive oxygen species

The methods in detail were described as in our previous studies [4,7]. Cultured arterial smooth muscle cells (A10) were loaded with MitoSOX (2 μM) for 20 min and Hoechst (1 μg/mL) for 15 min