Vascular Pharmacology 49 (2008) 26–31

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Antihypertensive and vasorelaxant activities of *Laelia autumnalis* are mainly through calcium channel blockade ☆

Jorge Vergara-Galicia [a], Rolffy Ortiz-Andrade [a], Patricia Castillo-España [b], Maximiliano Ibarra-Barajas [c], Itzell Gallardo-Ortiz [c], Rafael Villalobos-Molina [c], Samuel Estrada-Soto [a,c,*]

[a] Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Colonia Chamilpa, C.P. 62209. Cuernavaca, Morelos, Mexico
[b] Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Colonia Chamilpa, C.P. 62209. Cuernavaca, Morelos, Mexico
[c] Unidad de Biomedicina, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, Mexico, Mexico

ARTICLE INFO

Article history:
Received 21 September 2007
Received in revised form 9 April 2008
Accepted 18 April 2008

Keywords:
Aortic rings
Calcium channel
Laelia autumnalis
Orchid
Vasorelaxant

ABSTRACT

The aim of the present study was to evaluate the possible mechanism of the vasorelaxant action of methanol extract from *Laelia autumnalis* (ME*La*) in isolated rat aortic rings, and to establish its antihypertensive activity *in vivo*. ME*La* (0.15→50 μg/mL) induced relaxation in aortic rings pre-contracted with KCl (80 mM), showing an IC_{50} value of 34.61 ± 1.41 μg/mL and E_{max} value of 85.0 ± 4.38% (in endothelium-intact rings) and an IC_{50} value of 45.11 ± 4.17 μg/mL and E_{max} value of 80.0 ± 12.1% (in endothelium-denuded rings). Serotonin (5-HT, 1×10^{-4} M) provoked sustained contraction, which was markedly inhibited by ME*La* (0.15→50 μg/mL) in a concentration-dependent and endothelium-independent manner. Pretreatment with ME*La* (15, 46, 150, 300 and 1500 μg/mL) also inhibited contractile responses to norepinephrine (NE 1×10^{-11} M to $1 \times 10^{-5.5}$ M). In endothelium-denuded rings, the vasorelaxant effect of ME*La* was reduced partially by ODQ (1 μM), but not by tetraethylammonium (5 μM), glibenclamide (10 μM), and 2-aminopyridine (100 μM). The extract also reduced NE-induced transient contraction in Ca^{2+}-free solution, and inhibited contraction induced by increasing external calcium in Ca^{2+}-free medium plus high KCl (80 mM). The antihypertensive effect of ME*La* was determined in spontaneously hypertensive rats (SHR). A single oral administration of the extract (100 mg/kg) exhibited a significant decrease in systolic and diastolic blood pressure and heart rate ($p<0.05$) in SHR rats. Our results suggest that ME*La* induces relaxation in rat aortic rings through an endothelium-independent pathway, involving blockade of Ca^{2+} channels and a possible cGMP enhanced concentrations and also causes an antihypertensive effect.

1. Introduction

Hypertension, defined as an elevation of systolic and/or diastolic blood pressure to above 140/90 mm Hg, is the most common cardiovascular disease. Hypertension is a major risk factor for endothelial dysfunction, metabolic syndrome, diabetes, renal dysfunction, congestive heart failure, coronary artery disease and stroke (Ogihara et al., 2005). These diseases are the most important causes of death in the world. Antihypertensive drugs influence arterial blood pressure at four effectors' sites: the resistance vessels, the capacitance vessels, the heart, and the kidney. Important classes of antihypertensive drugs are diuretics, sympatholytic drugs, vasodilators, calcium channel blockers and angiotensin converting enzyme inhibitors (Staffileno, 2005). Primary hypertension can no longer be considered simply as a state of elevated blood pressure; it is a syndrome of multiple abnormalities often characterized by cardiac and vascular remodeling, lipid abnormalities and defects in carbohydrate metabolism associated with insulin resistance/hyperinsulinemia (Weber et al., 1992). New antihypertensive therapy is needed to control hypertension more effectively, with fewer side effects and neutral impact on known cardiovascular risk factors (Cogolludo et al., 2005). Required morbidity-mortality trials should be initiated early to establish the advantages of new agents. In an attempt to found novel antihypertensive compounds with vasorelaxant activity, we started a project to study medicinal plants that are used as antihypertensive agents and related diseases in Mexican traditional medicine. In this context, many Mexican regions show a high herbal diversity of medicinal plants, representing a considerable source to study their pharmacology and chemical properties for the discovery of new drugs. The Orchidaceae family, including the *Laelia* genus, has more than 30,000 species widely distributed in the world. *Laelia autumnalis* is an endemic

☆ This work was taken in part from PhD Thesis of Jorge Vergara-Galicia and sabbatical stay of Dr. S. Estrada-Soto.

* Corresponding author. Laboratorio L-12 de Farmacognosia y Química de Productos Naturales, Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Colonia Chamilpa, C.P. 62209, Cuernavaca, Morelos, Mexico. Tel./fax: +52 777 3 29 70 89.
E-mail address: enoch@uaem.mx (S. Estrada-Soto).

1537-1891/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2008.04.002

medicinal species in Mexico, and it is used for the treatment of diarrhea and abortion (Castillo–España P. and Monroy-Ortiz, 2000). There are no ethnopharmacological reports of *L. autumnalis* being used as an antihypertensive agent, but in a previous investigation, we showed that the methanolic extract from *L. autumnalis* possesses a significant vasorelaxant activity on contraction induced by norepinephrine (NE, $1\times10^{-7.5}$ M) in endothelium-independent and concentration-dependent manners on isolated rat aorta rings (Aguirre-Crespo et al., 2005). Nevertheless, the pharmacological properties of *L. autumnalis* have not yet been widely studied. Therefore, the aim of the present study was to characterize the possible mode of vasorelaxant action, and to show its antihypertensive property with the purpose to find new compounds that produce vasodilatation.

2. Materials and methods

2.1. Chemicals and drugs

Carbamoylcholine HCl (carbachol), norepinephrine HCl (NE), 5-hydroxytryptamine creatinine sulphate (5-HT), nitrendipine, 1-*H*-[1,2,4]-oxadiazolo-[4,3a]-quinoxalin-1-one (ODQ), glibenclamide, tetraethylammonium (TEA) and 2-aminopyridine (2-AP) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other reagents were analytical grade from local sources. For *in vitro* experiments, ME*La* was dissolved in distilled water and DMSO (1% v/v), while that glibenclamide was dissolved in DMSO and the other reagents were dissolved in distilled water and sonicated just before use. Preliminary experiments showed that DMSO at 1% (v/v) had no effect on tension development of isolated aorta. For *in vivo* experiments, ME*La* was dissolved in Tween 80 (2%), brought to the chosen volume with sterile isotonic saline and sonicated just before use.

2.2. Plant material and extraction

L. autumnalis plant material was collected on November 2004 in Tepoztlán, Morelos, Mexico. The identification and collection of the plant was carried out by Dr. Patricia Castillo-España. A voucher specimen (No. 22025) was deposited at the HUMO-Herbarium of the Centro de Estudios Ambientales e Investigación "Sierra de Huautla" (CEAMISH) of the Morelos State University. Briefly, the dried plant material was pulverized (370 g) and crude extracts were prepared by successive maceration with MeOH (3 times for 72 h at room temperature). After filtration, extracts were concentrated *in vacuo* at 40 °C. Finally, a yield of 50.70 g of crude methanolic extract was obtained.

2.3. Animals

To determine the vasorelaxant effect and the mode of action of ME*La* male Wistar rats (250–350 g) were used, and for evaluation of the antihypertensive activity male Spontaneously Hypertensive Rats (SHR) (250–350 g) were used. They were maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted in accordance with our Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZO-1999, Mexico) and were approved by the Institutional Animal Care and Use Committee.

2.4. Preparation of rat aortic rings

Rats were sacrificed by exposure to ether and the thoracic aortas were removed and immersed in Krebs solution at room temperature. Aortic rings (3–5 mm) were obtained free from connective tissue and fat (in some rings the endothelium was removed), and suspended by platinum hooks under an optimal tension of 3 g in a Krebs solution (composition, mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; EDTA, 0.026; glucose, 11.1, pH 7.4), maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2. Isometric tension was measured and recorded using Grass-FT03 force transducers (Astromed®, West Warwick, RI, USA) connected to a MP100 analyzer (BIOPAC® Instruments, Santa Barbara, CA, USA).

In order to establish the mode of action of ME*La*, six sets of experiments were conducted:

2.4.1. Effect of MELa on the contraction induced by 5-HT and KCl

The aortic rings with and without endothelium were precontracted with 5-HT (1×10^{-4} M) and high KCl (80 mM). Once the plateau was attained, concentration–response curves of ME*La*-induced relaxation (0.15 μg/mL→50 μg/mL) were obtained by adding cumulative concentrations of the extract to the bath.

2.4.2. Effect of MELa on the contraction induced by NE

Endothelium-denuded aortic rings were incubated with 15, 46, 150 y 300 μg/mL of ME*La* during 15 min, then NE was added at different concentrations (1×10^{-11} M→$1\times10^{-5.5}$ M). Finally, the contractile effect induced by NE was compared in absence (control group) and presence of the extract.

2.4.3. Effect of MELa on extracellular Ca^{2+}-induced contraction activated by KCl

To determine whether the inhibition of extracellular Ca^{2+} influx was involved in ME*La*-induced relaxation, the experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded aortic rings were washed with Ca^{2+}-free solution (approximately 20 min) and then rinsed with Ca^{2+}-free solution containing KCl (80 mM). The cumulative concentration–response curves for $CaCl_2$ (1×10^{-4} M→0.01 M) were obtained in the absence of ME*La* (control group) or after a 15 min incubation with the extract (34 or 100 μg/mL). Finally, the contractile effect induced by $CaCl_2$ was compared in absence (control group) and presence of ME*La*.

2.4.4. Effect of MELa on the sarcoplasmic reticulum calcium release induced by NE

To clarify whether the relaxation induced by the extract was related to inhibition of intracellular Ca^{2+} release, the experiments were carried out in Ca^{2+}-free Krebs solution. Endothelium-denuded rings were washed with Ca^{2+}-free Krebs solution during 10 min, then ME*La* (34 or 100 μg/mL) was present during 20 min and finally, NE (1×10^{-7} M) was added to stimulate the release of intracellular Ca^{2+}. The maximal tension induced by NE in the control group (without ME*La*) was considered as 100%.

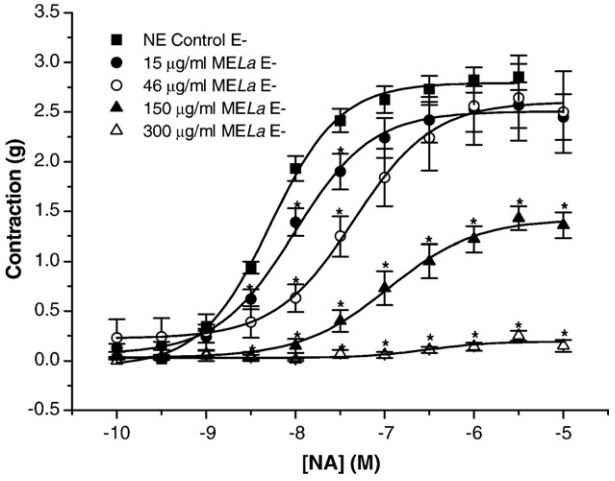

Fig. 1. Inhibitory effects of ME*La* on the contraction induced by NE (1×10^{-11} to $1\times10^{-5.5}$ M) in endothelium-denuded aortic rings. Results are presented as mean ± S.E.M. $n=6$, $^*p<0.05$ compared with control.