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ScienceDirect
Vascular Pharmacology 46 (2007) 60–66
Vascular Pharmacology
www.elsevier.com/locate/vph

Mechanisms underlying the cardiovascular effects of a labdenic diterpene isolated from *Moldenhawera nutans* in normotensive rats

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Received 1 March 2006; received in revised form 7 May 2006; accepted 14 June 2006

Abstract

Cardiovascular effects of Labd-8 (17)-en-15-oic acid (Labd-8), a labdenic diterpene isolated from methanolic extract of *Moldenhawera nutans* were investigated in normotensive rats. Additionally, this study examined the role of autonomic nervous system in the mediation of these cardiovascular effects. In pentobarbital-anesthetized rats, bolus intravenous (i.v.) injection of Labd-8 (1–10 mg/kg) induced dose-dependent hypotensive and tachycardiac responses. After cervical bivagotomy, hypotensive responses to Labd-8 were significantly enhanced whereas the tachycardia was completely abolished. In conscious rats, Labd-8 (1–10 mg/kg, i.v.) also decreased blood pressure and increased heart rate in a dose-dependent manner. Pretreatment with methylatropine (1 mg/kg, i.v.) or propranolol (2 mg/kg, i.v.) significantly reduced the tachycardia evoked by Labd-8 without affecting the hypotension. Blockade of ganglionic neurotransmission with hexamethonium (30 mg/kg, i.v.) reduced and abolished the hypotensive and tachycardic effects of Labd-8, respectively. However, hypotensive effects of Labd-8 were not reduced by pretreatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg, i.v.), a nitric oxide synthase inhibitor. In rat endothelium-containing aorta preparations, Labd-8 (1–1000 μg/ml) induced a concentration-dependent reduction of potassium (60 mM)-induced contraction [IC_{50} (geometric mean ±95% confidence interval)=313.6 (191.4–513.8) μg/ml], an effect that remained unaffected [IC_{50}=440.8 (225.1–863.3) μg/ml] by removal of vascular endothelium. These results show that i.v. treatment with Labd-8-induced dose-dependent hypotensive and tachycardiac effects in both conscious and anesthetized rats. The tachycardia is mediated reflexly through inhibition of vagal and activation of sympathetic drive to the heart. The hypotension is mainly due to withdrawal of sympathetic tone to the vasculature and also partly to an active vascular relaxation. Released nitric oxide from vascular endothelial cells is not involved in the mediation of Labd-8-induced hypotension.

Keywords: Autonomic nervous system; Cardiovascular effects; Isolated thoracic aorta; Labd-8(17)-en-15-oic acid; Leguminosae; *Moldenhawera nutans*; Rat

1. Introduction

Moldenhawera is a neotropical genus and it is distributed almost exclusively at the north coast of Brazil mainly in the Bahia State. It is represented by approximately 10 species (Queiroz et al., 1999). The taxonomy of this genus is confusing, making the classification of the species difficult (Lewis, 1987). *Moldenhawera nutans* Queiroz and Alkin (Leguminosae) is a shrub apparently endemic of the coastal dunes of Salvador, State of Bahia. In a former phytochemical study with the stems of this species were reported the isolation and identification of several labdenic diterpenes (David et al., 1998), including labd-8(17)-en-15-oic acid (Labd-8), 3β,15-dihydroxy-labd-8(17)-ene, 19-hydroxy-labd-8(17)-en-15-oic acid, and 3β-dihydroxy-labd-8 (17)-en-15-oic acid. None of these identified pure diterpenes showed the same activity exhibited by the methanolic extract (72% at 200 μg/ml) of *M. nutans* in the HIV-1 RT (p 66) assay

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1537-1891/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2006.06.010

(David et al., 1998). Very few studies have been addressed with *M. nutans*. Recently, a novel flavonol glycoside compound, laricetrin 5-gallaoy-3-β-D-xylopyranoside, has been isolated from the ethyl acetate extract of *M. nutans* leaves (do Vale et al., 2005). Unlike laricetrin, this novel compound showed any significant antioxidant activity in the 1,2-diphenyl-2-picryl-hydrazyl (DPPH) radicals scavenging test (do Vale et al., 2005).

The present investigation, which is comprised of two parts, was undertaken to assess the cardiovascular effects of Labd-8 (Fig. 1), the major labdenic diterpene found in the methanolic extract of *M. nutans* in rats (David et al., 1998). The first part was performed in conscious or anesthetized rats in order to assess the cardiovascular effects of Labd-8 and the role of the autonomic nervous system as well as the endothelial L-arginine/nitric oxide (NO) pathway in the mediation of these effects. The second one was performed in vitro, using rat isolated thoracic aorta to assess whether the hypotensive effects of bolus injections of Labd-8 could result, at least in part, from its vasodilatory effects directly upon vascular smooth muscle.

2. Materials and methods

2.1. Plant material

Stems parts (2.1 kg) of *M. nutans* were collected on November 2000, at the Metropolitan Park area of Abaeté, Salvador, State of Bahia. Identification of the plant was confirmed by Prof. Maria Lenise da Silva Guedes (Institute of Biology, Federal University of Bahia). A voucher specimen (no. 029057) is deposited at the Alexander Leal Costa Herbarium, Institute of Biology, Federal University of Bahia. Labd-8 was extracted and isolated from the dried plant material according to previously reported procedures (David et al., 1998). Briefly, the dried plant material was first submitted to maceration with MeOH and the crude methanolic extract obtained (270 g) was immediately partitioned between hexane:MeOH/H_2O (9:1) and $CHCl_3$:MeOH/H_2O (6:4). The hexane partition (43.2 g) was then fractionated by gel silica column chromatography (CC) using hexane/EtOAc 95:5 as eluent. These procedures permitted to obtain the pure diterpene Labd-8 (12.5 g) from the second and third fractions (125 ml) eluted from the CC. The structure of Labd-8 was identified by spectral analysis and the unequivocal assignment of NMR data was based on bidimensional experiments (David et al., 1998). The purity of Labd-8 was 99.5% w/w as determined by GC/MS analysis.

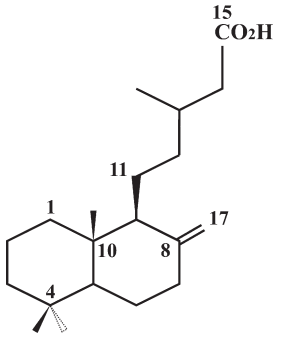

Fig. 1. Chemical structure of Labd-8(17)-en-15-oic acid.

2.2. Animals

Male Wistar rats were obtained from our local colonies maintained at the Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife, Brazil. They were kept under conditions of constant temperature (22±2 °C) with a 12-h light–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; http://www.nap.edu/readingroom/books/labrats/index.html) and had prior approval from the local animal ethics committee.

2.3. In vivo experiments

Rats (250–320 g) were anesthetized with intraperitoneally (i.p.) injected sodium pentobarbital (50 mg/kg supplemented by an additional 15 mg/kg when necessary), and catheters (PE-10 fused to PE-50) were implanted in the abdominal aorta (for the recording of arterial blood pressure) and in the inferior vena cava (for drug administration) through the left femoral artery and vein, respectively. These catheters, filled with heparin–saline solution (125 IU/ml), were exteriorized at the dorsal neck level. Postoperatively, the rats received an intramuscular injection of penicillin (24,000 IU), housed individually in plastic cages and allowed to recover for 24–48 h before any circulatory experiments. At the time of an experiment, the arterial catheter was connected to a blood pressure transducer (Statham P23 ID) coupled to a polygraph recorder; heart rate (HR) was obtained from a cardiotachometer triggered by the pressure pulses. Both signals were recorded on a Gilson model 5/6H polygraph (Medical Electronics Inc., Middletown, WI, USA). Mean aortic pressure (MAP) was calculated as diastolic + [(systolic − diastolic)/3].

Before each experiment, blood pressure and HR were allowed to stabilize and were recorded during 5–10 min (according to the duration of effects) after i.v. treatment with Labd-8. When subsequent doses of Labd-8 were administered, MAP and HR were first allowed to return to their baseline levels, obtained before the first injection of the drug. When the effects of an antagonist were tested, antagonist injection occurred 10 min before Labd-8 administration. Only one antagonist was administered to any individual animal per day. Two series of experiments were performed as follows:

Series 1: This series of experiments was carried out in anesthetized rats to establish a dose–effect relationship. Rats were again anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The body temperature was maintained by an electric heating pad. After a stable MAP and HR was obtained, each animal received a series of increasing bolus (100 μl) doses of Labd-8 (1, 3, 5 and 10 mg/kg), via the i.v. catheter, and time-course of the changes in MAP and HR was recorded. These experiments were performed in both intact rats ($n=6$), and in rats ($n=7$) that had been subjected to a bilateral vagotomy performed at the cervical level 15 min earlier. In both intact ($n=3$) and bivagotomized ($n=3$)