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Pharmacological Research 54 (2006) 150–157

Pharmacological research

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Vasorelaxant effects of harmine and harmaline extracted from *Peganum harmala* L. seed's in isolated rat aorta

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Accepted 7 April 2006

Abstract

The present work describes the mechanisms involved in the vasorelaxant effect of harmine and harmaline. These alkaloids induce in a dose-dependent manner the relaxation in the aorta precontracted with noradrenaline or KCl. However, the removal of endothelium or pre-treatment of intact aortic ring with L-NAME (inhibitor of NOSe synthetase) or with indomethacin (non-specific inhibitor of cyclo-oxygenase), reduces significantly the vasorelaxant response of harmaline but not harmine. According to their IC_{50} values, prazosin (inhibitor of α-adrenorecepteors) reduces the vasorelaxant effect only of harmaline, whereas, pre-treatment with IBMX (non-specific inhibitor of phosphodiesterase) affects both the harmaline and harmine-responses. Inhibitions of L-type voltage-dependent Ca^{2+} channels (VOCs) in endothelium-intact aortic rings with diltiazem depress the relaxation evoked by harmaline as well as by harmine. Pre-treatment with harmaline or harmine (3, 10 or 30 μM) shifted the phenylephrine-induced dose response curves to the right and the maximum response was attenuated indicating that the antagonist effect of both alkaloids on α_1-adrenorecepteors was non-competitive. These two alkaloids also exert an antioxidant activity by scavenging the free radical generated by DPPH. Therefore, the present results suggest that the vasorelaxant effect of harmaline but not harmine is related to its action on the prostacyclin pathway and on the endothelial cells to release NO. However, both alkaloids can act as blockers VOCs, as inhibitors of phosphodiesterase resulting in an increase of the second messenger (cAMP and cGMP) levels and finally reduce the levels of free radicals in tissues.
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Keywords: *Peganum harmala* L.; Harmaline; Harmine; Rat aorta; Vasorelaxant response; Antioxidant; Endothelium

1. Introduction

Peganum harmala L. (Zygophyllaceae), known as *harmel*, grows spontaneously in uncultivated and rocky areas from Mediterranean region (semiarid region) [1]. *Peganum harmala* is used in traditional medicine and is rich in alkaloids that have a wide spectrum of pharmacological actions in various areas. These include antispasmodic, antipyretic [2,3], anticancerous [4], central nervous system effects [5], hallucinogenesis [6], central monoamine oxidase inhibition [7], binding to various receptors including 5-HT and the benzodiazepine-binding receptors [8], platelet aggregation inhibitory [9] and immunomodulatory effects [10]. Ethnopharmacological observations have reported the hypotensive effects of *Peganum harmala* [1], also Aarons et al. [11], have demonstrated that harmala alkaloids include systemic arterial blood pressure reduction. However, the cellular and molecular mechanisms by which these alkaloids exert their actions remains unclear even though recent studies have elucidated in part the mechanism of action related to the vasorelaxant effects of synthetic β-carboline harmala-alkaloids [12,13].

We recently reported the vasorelaxant effect of a methanolic extract from seeds of *Peganum harmala* (MEP); our results suggested that the vasodilatory effect of this extract is endothelium-independent and is related to the inhibition of cyclic-AMP phosphodiesterase [14]. The present study

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doi:10.1016/j.phrs.2006.04.001

extends the exploration of the vasorelaxant-mechanism of harmala-alkaloids and compares the mechanism-pathways between natural compounds extracted from the plants and their synthetic form as proposed by Shi et al. [13].

2. Material and methods

2.1. Chemical reagents and drugs

Acetylcholine chloride, noradrenaline (bitartrate salt), phenylephrine hydrochloride, diltiazem, sodium nitroprusside, N^{ω}-nitro-L-argenine methyl ester (L-NAME) and indomethacin were purchased from Sigma Chemical Co. (St. Louis, USA) and dissolved in distilled water. Prazosin (Pfizer), IBMX (3-isobutyl,1-methylxanthine), Sigma Chemical Co. (St. Louis, USA), were dissolved in dimethylsulfoxide (DMSO). The final DMSO concentrations (0.001%) did not significantly affect the results. Ascorbic acid (10^{-4} M) was added to fresh noradrenaline solutions to prevent possible oxidation.

2.2. Plant

Peganum harmala L. (Zygophyllaceae) fresh seeds were collected from the Atlas region of Morocco, in May 2000 and botanically identified by the botanical section of U.F.R: Naturals Products, Faculty of Medicine and Pharmacy (Rabat), where a specimen is preserved (number PH-00052).

2.3. Extraction of natural compound

Fresh and powdered seeds were prepared as previously described (Berrougui et al. [14]). The natural compounds (harmaline and harmine) were extracted from the last fraction (methanolic extract) as fellow: briefly, methanolic extract was eluted on a silica gel column initially with pure chloroform ($CHCl_3$), and then increasing amounts of methanol (CH_3OH). All fractions obtained from this silica gel column were subjected to thin layer chromatography (TLC) examination using $CHCl_3$–MeOH (9:1). Same fractions were pooled and the harmine and harmaline were washed successively in $CHCl_3$ and MeOH, crystallised and then analysed by TLC, ^{1}H NMR, ^{13}C NMR (Nuclear Magnetic Resonance) and mass spectrometry.

2.4. Animals

Wistar rats weighing 100–120 g, were purchased from Harlan Ibérica (Barcelona, Spain). All experiments were performed according to guidelines for the ethical treatment of animals of the European Union (86/609/EEC). All rats were fed with standard rat chow (Panlab SRL, Barcelona, Spain) and maintained in a temperature-controlled room ($24 \pm 2\,^{\circ}C$) with $60 \pm 20\%$ relative humidity, a 12 h light–dark cycle and with free access to standard rat chow and drinking water. All experiments were performed on 12–14-week-old rats. The animals were sacrificed by cervical dislocation and the aorta was rapidly dissected.

2.5. Aortic ring preparation

The descending thoracic aorta was placed in a modified Krebs-Henseleit solution (PSS) containing (mM), NaCl (118), KCl (4.75), $NaHCO_3$ (25), $MgSO_4$ (1.2), $CaCl_2$ (1.8), KH_2PO_4 (1.2) and glucose (11). After excess fat and connective tissues were removed, the aortas were cut into 2–3-mm rings. Aortic rings were mounted under a basal tension of 2 g in 20 ml organ baths containing PSS as previously describe (Herrera-Gonzalez et al., 1996) and attached to an isometric transducer (harvard UF-1), the signal was recorded by powerlab® data acquisition system (AD-Instruments). The tissue bath was maintained at 37 °C and bubbled with a 95% O_2–5% CO_2 gas mixture. In some experiments, the endothelium was mechanically removed by gently rubbing the inner surface of the rings. The absence of endothelium E(−) was then verified by addition of acetylcholine (ACh 10^{-6} M) in aortic rings previously contracted by phenylephrine (Phe 10^{-6} M). Each preparation was allowed to equilibrate for at least 60-min prior to initiation of experimental procedures, and during this period the incubation media was changed every 20 min [15].

After equilibration, the following experiments were performed:

To determine whether harmine and harmaline could relax an existing contraction, aortic rings were contracted by a single sub-maximal concentration of noradrenaline (NA 10^{-6} M) or KCl (80 mM). When the contractile response to either agonist was stable, harmine or harmaline were added in progressively increasing cumulative concentrations (1–300 μM) at 20-min intervals (time interval necessary to done the maximal effect of extract: plate of action). Harmaline was dissolved in the PSS, whereas, harmine was in the DMSO with final DMSO-concentrations (0.001%) that did not significantly affect the results. The results were expressed as a percentage of the maximal control agonist-induced response.

The involvement of the endothelium-related vasorelaxation in the harmine and harmaline-induced relaxation was examined in intact aortic ring pre-treated with nitric oxide synthase-inhibitor, L-NAME (30 μM) or with non-selective cyclooxygenase inhibitor, indomethacin (10 μM) [13].

To investigate the effects of these alkaloids on the endothelium-independent relaxation, endothelium-denuded aortic preparations E(−) were incubated for 30 min with harmine or harmaline (10^{-5} M) prior to the precontraction with phenylephrine (10^{-6} M), and when the contraction was stabilised, cumulative concentrations of sodium nitropruside (10^{-10} to 10^{-7} M, nitric oxide donor) were added.

In another experiment, intact aorta was treated with an α-adrenoreceptors inhibitor, prazosin (10^{-8} M) or a non-specific phosphodiesterase-inhibitor, IBMX (10^{-4} M). Cumulative concentrations of harmine and harmaline were added following the stabilisation phase of KCl-induced contraction.

The effect of alkaloids on the VOCs was studied in the aortic ring previously treated during 20 mn with a voltage-dependent Ca^{2+}-channels inhibitor, diltiazem (10^{-6} M). Preparation ring was then contracted by noradrenaline followed by the adding of cumulative concentration of harmine or harmaline were added.

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