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Pre-treatment with α-hederin increases β-adrenoceptor mediated relaxation of airway smooth muscle

Anne Wolf[a], Reinoud Gosens[b], Herman Meurs[b], Hanns Häberlein[a,*]

[a] Institute of Biochemistry and Molecular Biology, Rheinische Friedrich-Wilhelms-Universität, Nussallee 11, 53115 Bonn, Germany
[b] Department of Molecular Pharmacology, University Groningen, A. Deusinglaan 1, 9713 AV, Groningen, The Netherlands

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ABSTRACT

Preparations of ivy leaves dry extract with secretolytic and bronchiolytic efficacy are widely used for the treatment of acute and chronic obstructive airway diseases. The mechanism by which ivy preparations improve lung functions is not fully understood. Here, we tested the influence of the three main saponins of ivy, α-hederin, hederacoside C and hederagenin, on the contraction and relaxation behaviour of isolated bovine tracheal smooth muscle strips by isometric tension measurements. None of the tested compounds altered histamine or methacholine-induced contraction of the smooth muscle strips. In contrast, the isoprenaline-induced relaxation of 100 μM methacholine precontracted muscle strips was significantly enhanced when pre-treated with 1 μM of α-hederin for 18 h. The pre-treatment with hederacoside C or hederagenin had no effect on isoprenaline-induced relaxation. For the first time the bronchiolytic effect of α-hederin was demonstrated by isometric tension measurements using bovine tracheal smooth muscle strips. α-Hederin increases isoprenaline-induced relaxation indirectly, probably by inhibiting heterologous desensitization induced by high concentrations of muscarinic ligands like methacholine.

Introduction

Preparations of ivy leaves dry extract are used for the therapy of acute and chronic obstructive pulmonary diseases characterized by hypersecretion of viscous mucus and coughing. Their efficacy and very good tolerability have been confirmed by controlled clinical trials and post-marketing surveillance studies (PMSS) in both, adults (Meyer-Wegener et al., 1993) and children (Gulyas et al., 1997; Mansfeld et al. 1997; Hofmann et al. 2003; Fazio et al. 2009) suffering from acute or chronic obstructive bronchitis. Both spirometric as well as plethysmographic investigations showed clinically relevant improvement of lung function parameters after therapy with an ivy leaves dry extract preparation (drug to extract ratio (DER) = 5–7.5:1, extracting medium: ethanol 30%) which therefore is by now registered in more than 70 countries worldwide. Saponins are considered as the active compounds in ivy leaves extracts and preclinical studies on living cells analysing the dynamics and regulation of β_2-adrenoceptors (β_2AR) recently identified α-hederin as the main active constituent (Fig. 1) (Sieben et al., 2009). α-Hederin pre-treated alveolar type II cells (A549) showed an increased β_2AR binding caused by a higher binding affinity, whereas the maximum number of binding sites (B_{max}) was not affected in saturation experiments compared to untreated control cells (Sieben et al., 2009). In HEK 293 cells, overexpressing β_2AR as a GFP fusion protein, preincubation with α-hederin inhibited the internalization of β_2AR-ligand complexes even after stimulation with terbutaline (Sieben et al., 2009). Thus, one can expect an elevated β_2-adrenergic responsiveness under stimulating conditions and a subsequently increased signal transduction which was found for α-hederin pre-treated human airway smooth muscle cells (HASM) after stimulation with terbutaline and forskolin (Sieben et al., 2009).

In this paper, we describe the influence of the major saponins α-hederin, hederacoside C, and hederagenin from ivy leaves dry extract on the contraction and relaxation behaviour of isolated bovine tracheal smooth muscle strips.

Methods

Tissue preparation

Fresh bovine tracheas obtained from local slaughterhouses were transported to the laboratory within 30 min after removal at room temperature in Krebs–Henseleit (KH) buffer of the following composition (nM): NaCl 117.5, KCl 5.6, $MgSO_4$ 1.18, $CaCl_2$ 2.52, NaH_2PO_4 1.28, $NaHCO_3$ 25.0 and glucose 5.55, pregassed with 5% CO_2 and 95% O_2 (pH 7.4). Tracheal smooth muscle strips free of mucosa and serosal connective tissue were prepared after dissec-

* Corresponding author. Tel.: +49 228 736555; fax: +49 228 732416.
E-mail address: haeberlein@uni-bonn.de (H. Häberlein).

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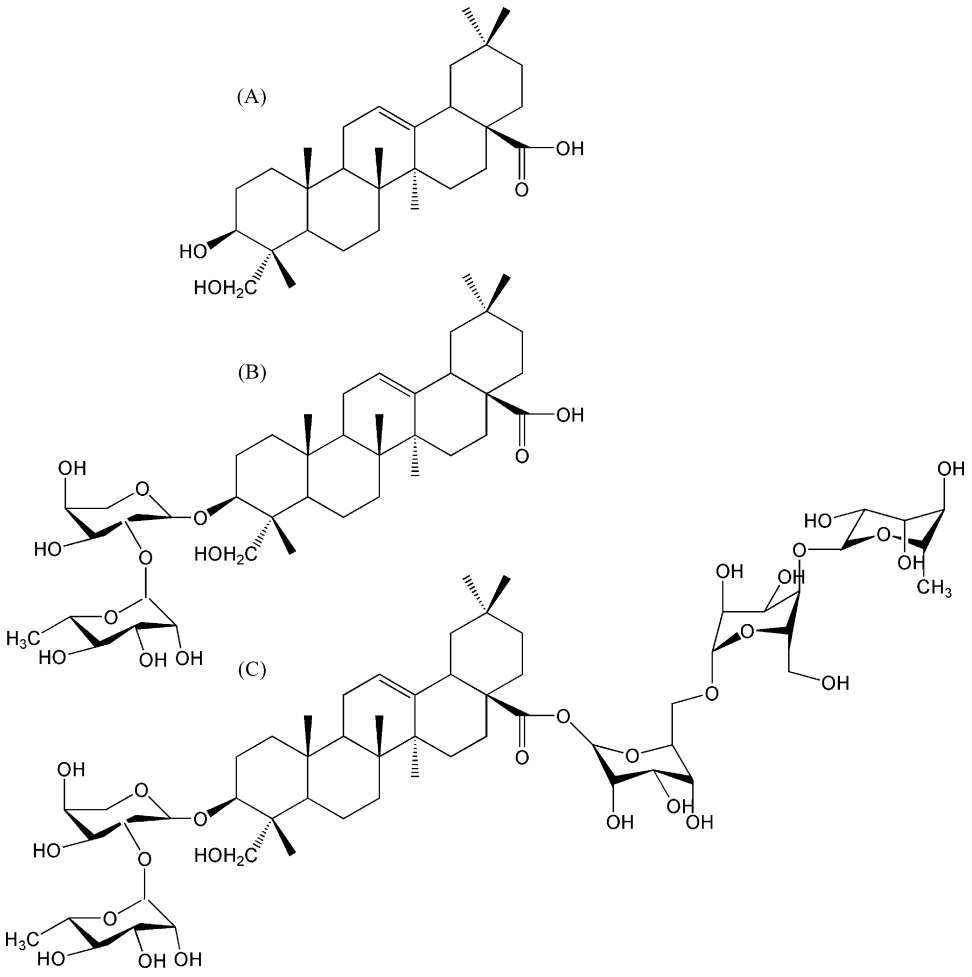

Fig. 1. Structures of hederagenin (A), α-hederin (B) and hederacoside C (C).

tion of the muscle in KH buffer gassed with 5% CO_2/95% O_2 at room temperature. All strips were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 μg/ml streptomycin, 100 U/ml penicillin, 50 μg/ml gentamicin, 1.5 μg/ml amphotericin B, 1 mM sodium pyruvate, and a non-essential amino acid mixture for 1–3 days at 37 °C and 55 rpm. 18 h prior to the experiments 1 μM α-hederin, 1 μM hederacoside C, 1 μM hederagenin or 0.1% ethanol (as vehicle) were added to the media containing the strips.

Isometric tension measurements

Tissue strips, collected from suspension culture flasks, were washed with several volumes of KH-buffer pregassed with 5% CO_2 and 95% O_2, pH 7.4 at 37 °C. Subsequently, strips were mounted for isometric recording (Grass force-displacement transducer FT03) in 20-ml water-jacked organ baths, containing KH-buffer at 37 °C, continuously gassed with 5% CO_2 and 95% O_2, pH 7.4 and the adequate saponin or ethanol as vehicle. During a 90 min equilibration period, with washouts every 30 min, resting tension was gradually adjusted to 3 g. Subsequently, muscle strips were precontracted with 20 and 40 mM isotonic KCl solutions. Following two washouts, tension was re-adjusted to 3 g, immediately followed by two changes of fresh KH-buffer.

For the contraction experiments dose–response curves were constructed by increasing concentrations of histamine from 1 nM to 100 μM in the organ bath or in the case of methacholine by contraction of muscle strips with 1 and 100 μM methacholine, respectively. At the end of the experiment, basal tone was re-established by washing several times. For the relaxation experiments strips were precontracted with varying concentrations of methacholine (1–100 μM) or 100 μM histamine followed by relaxation via increasing concentrations of isoprenaline (1 nM to 100 μM). After the experiments muscle strips were weighed and force was related to muscle mass.

Chemicals

Dulbecco's modified Eagle's medium, fetal calf serum, streptomycin, penicillin, gentamicin, amphotericin B (Fungizone®), sodium pyruvate and non-essential amino acid mixture were from Invitrogen (Carlsbad, CA, USA). Histamine and isoprenaline were from Sigma Chemical Co. (St Louis, MO, USA), methacholine from ICN Biomedicals (Costa Mesa, CA, USA), α-hederin from Carl Roth (Karlsruhe, Germany), hederacoside C and hederagenin from Extrasynthèse (Genay Cedex, France). All other chemicals were from Merck (Darmstadt, Germany).

Data analysis

Responses for the contraction experiments were expressed as percentage of maximum contraction and for the relaxation experiments as percentage of the response to the used methacholine concentration. EC_{50} values are calculated from the dose–response curves by the dose–response function for agonist of GraphPad Prism 5.02 (GraphPad Software, Inc.). All data represent