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Inhibition of histamine-induced bronchospasm in guinea pigs treated with *Cecropia glaziovi* Sneth and correlation with the in vitro activity in tracheal muscles

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Abstract

A standardized aqueous extract (AE) and a purified fraction (BuF) of *Cecropia glaziovi* Sneth leaves were tested in unrestrained guinea pigs challenged with histamine. Changes of the respiratory pressure and rate were recorded in a whole body plethysmograph before and after treatment. The concentration of histamine necessary to produce bronchospasm was increased by five-fold following administration of AE (1.0 g/kg *p.o.*), and by two-fold after treatment with the semi-purified procyanidin/flavonoids enriched BuF (0.1 g/kg *p.o.*). Both effects were blocked by previous treatment with propranolol (10.0 mg/kg *i.p.*). In vitro incubation of BuF (0.1–1.0 mg/ml) decreased by 13–55% the maximal response of guinea pig tracheal muscle to histamine, without significant change of EC_{50}. The results confirmed old reports on the useful pulmonary effects of Cecropia extracts. The bronchodilation observed in vivo seems to be related to β-adrenergic activity observed in vitro only with high concentrations of the purified extract.

Keywords: Histamine challenge; *Cecropia*; Medicinal plant; Phytotherapy; Bronchodilation

Introduction

Latin America's folk medicine refers the use of *Cecropia* sp. leaves extract in cough, asthma and bronchitis (Coimbra, 1958; Bahia, 1979). In vitro evaluation of Cecropia extracts and the effects induced after intravenous injection of crude extracts were reported (Sivori, 1933; Vieira et al., 1968; Vidrio et al., 1982; Lacaille-Dubois et al., 2001; Almeida et al., 2006; Consolini et al., 2006). An account of the plant cardiovascular and central nervous system pharmacology after oral administration was also presented (Lapa et al., 1999). None of these studies reported the extract effects on the respiratory system, except the preliminary work by Cysneiros (1996) using in vitro tracheal muscles. The plant chemistry has been studied and its content in procyanidins, catechins and flavonoids was reported (Lacaille-Dubois et al., 2001; Della Monache et al., 1998; Tanae et al., 2006). The effects of these constituents have been related to in vitro enzyme inhibition like the angiotensin-converting enzyme (Castro-Braga et al., 2000; Lacaille-Dubois et al., 2001) and the gastric H,K-ATPase (Souccar et al., 2006), and also to the hypotensive effects after i.v.

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injection (Lima-Landman et al., 2006), and smooth muscle relaxation (Lapa et al., 1999). The smooth muscle relaxation was attributed to calcium channel blockade (Lapa et al., 1999), but no other specific interaction was demonstrated upon i.v. injection or in vitro incubation of the crude extract and isolated compounds.

The present study describes the plant extracts effects on the respiratory system of unrestrained guinea pigs. Treatment *per os* with either the aqueous extract (AE) used in folk medicine, or the semipurified fraction (BuF) rich in catechins-procyanidins (25%) and flavonoids (22.5%) decreased the responses to histamine challenge. Evidences on the mechanism of tracheal musculature relaxation and inhibition of bronchospasm by the purified AE are also included.

Material and methods

All the experimental protocols were approved by the Institutional Ethical Committe (CEP-UNIFESP 293-00).

Animals and whole body plethysmography

Male adult guinea pigs (300–600 g) were used in all experiments. *Per os* drug administration used a metallic cannula carefully introduced into the stomach to deliver volumes never exceeding 3 ml. To record breathing from non-anesthetized animals, a 1.9 l whole body plethysmograph was built as described by Griffiths-Johnson and Karol (1991). Changes in pressure produced by breathing movements inside the chamber were recorded using a differential pressure transducer connected to a pen recorder.

Experimental protocol

After 15 min in the chamber the guinea pigs were challenged with saline followed by histamine (Hist), sprayed every 5 min at increasing concentrations (5–640 μg/ml) for 1 min; changes in the respiratory pressure were recorded during 3 min, or until bronchospasm occurred. Bronchospasm was defined when the amplitude of breathing pressure was increased by two-fold ($\Delta P \geqslant 2$) of the value recorded under basal conditions (Fig. 1). Challenging drugs were diluted in saline and sprayed inside the plethysmograph using a commercial ultrassonic sprayer (ICEL®). The 5 min interval between challenges was sufficient to record the animals' reactivity, to flush air into the chamber, to clear out the ineffective drug, and to record again the animals' basal breathing.

A few animals were previously sensitized with 0.7 ml ovalbumin (OVA) 5% in saline injected intraperitoneally (Popa et al., 1973). Twenty-one days afterwards these animals were challenged with 0.25% OVA sprayed during 20 s. Any animal non-reactive after 5 min challenge with OVA was discarded. On the following day all sensitized animals were treated with AE (1 g/kg *p.o.*) and challenged with OVA 2 h afterwards.

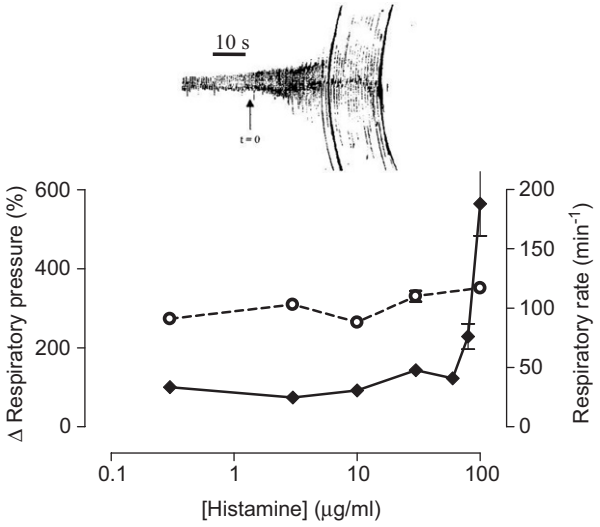

Fig. 1. Change of respiratory pressure (% of basal – ♦) and respiratory rate (breaths/min – ○) of unrestrained guinea pigs challenged with histamine. Bronchospasm occurred after spraying 80 and 100 μg/ml histamine, while the respiratory rate increased by 20%. The polygraphic recordings on the top, show a bronchospasm in a guinea pig challenged with histamine (100 μg/ml) sprayed at time zero. Bronchospasm was defined as a two-fold increase in the basal respiratory pressure. The symbols are means ± SE ($n = 6$).

Drugs

Cecropia AE were prepared as indicated in folk medicine: the ground leaves were extracted in hot water (2.0%, 72 °C) during 30 min, the AE was concentrated and freeze-dried (yield = 20%). Partition of AE in *n*-butanol yielded the purified fraction (BuF) used to isolate and identify the chemical constituents, as described elsewhere (Tanae et al., 2006). AE (1.0 g/kg) or BuF (0.1 g/kg) were administered *per os* 2 h before challenge. Ketotifen (1.0 mg/kg *p.o.*) or salbutamol (2.0 mg/kg *p.o.*) administered 1 h before Hist challenge were used as positive control (Ueno et al., 1998). Control animals received the equivalent volume of tap water.

Guinea pig tracheal rings

A chain of four tracheal rings was set in 2 ml organ bath containing Krebs, pH 7.4, at 37 °C. Isometric contractions elicited by histamine (10 nM–10 mM) were