

THE BRONCHORELAXANT EFFECT OF HELICIDINE, A *HELIX POMATIA* EXTRACT, INVOLVES PROSTAGLANDIN E₂ RELEASE

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ABSTRACT

Helicidine is a biological extract prepared from the snail Helix pomatia L. and used in man as an anti-tussive agent. However, its mechanisms of action are not fully defined. In this study, we have investigated a possible relaxant effect of helicidine on guinea-pig airway smooth muscle and evaluated the role of prostanoids and airway epithelium in this relaxation. H. pomatia extract (0.001–1 mg/ml) induced a dose-dependent relaxation of guinea-pig trachea pre-contracted with histamine both in the presence and absence of tracheal epithelium. No significant difference in dose-dependency or magnitude of the relaxation was observed between tracheal segments with or without epithelium (maximal relaxant response of 35 ± 7 and 25 ± 7.5%, respectively). Relaxation of the trachea induced by H. pomatia extract (0.001–1 mg/ml) was inhibited by pre-treatment with the cyclooxygenase inhibitor, indomethacin, both in the presence or absence of tracheal epithelium. H. pomatia extract (1 mg/ml) induced a marked and significant increase in prostaglandin E₂ release in tracheal segments with and without epithelium. These results indicate that helicidine possesses a bronchorelaxant activity which is independent of epithelium integrity and which is partly mediated by the release of the relaxant prostanoid, prostaglandin E₂. The origin of prostaglandin E₂ production in the airways remains to be defined.

Keywords: Airways, guinea-pig, helicidine, *Helix pomatia* L., prostaglandin E₂, prostanoids, relaxation.

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INTRODUCTION

Helicidine is a syrup commercialized in France and used in man as an anti-tussive agent. This drug is a biological extract prepared from the snail *Helix pomatia* L. (Mollusca: Gastropoda) (Quevauviller et al., 1953). Its mechanism of action has not been fully investigated. However, its anti-tussive property may not be the only mechanism involved in its therapeutic action.

Bronchopulmonary diseases are often associated with airway obstruction and inflammation, and with increased bronchial responsiveness to exogenous stimuli. Airway obstruction results not only from increased airway mucus secretion, but also from airway narrowing due to airway smooth muscle contraction. Besides mucolytic agents, drugs inducing bronchial smooth muscle relaxation represent one of the major classes of therapeutic agents used to attenuate airway obstruction. Therefore, we have investigated the effect of helicidine on bronchial smooth muscle, as a possible mechanism for its beneficial effect in pulmonary diseases.

In the airways, smooth muscle response to contractile and relaxant mediators is closely regulated by airway epithelium (Barnes et al., 1985; Farmer, 1987; Vanhoutte, 1987; Goldie et al., 1990). This regulatory role of epithelium involves its barrier function and its metabolic activity, which both limit the effect of agonists on airway smooth muscle. However, it also results from its capacity to release, upon stimulation, factors which either mediate or inhibit the effect of mediators on smooth muscle (Flavahan et al., 1985; Frossard et al., 1989, 1990; Frossard & Barnes, 1991).

Bronchorelaxant prostanoids derived from the cyclooxygenase metabolism of arachidonic acid, and in particular prostaglandin E₂, are among the principle substances released by epithelium upon agonist stimulation and involved in the mediation or the modulation

of agonist-induced smooth muscle response. Indeed, these products have been reported to modulate the response of guinea-pig trachea to contractile agents such as histamine, leukotriene C₄ or methacholine (Goldie et al., 1990), as well as to mediate the relaxant response induced by bradykinin (Frossard et al., 1990; Schlemper & Calixto, 1995). Moreover, prostaglandin E₂ has been shown to be involved in the relaxation of rat trachea induced by the tachykinin, substance P, through activation of specific epithelial receptors (Devillier et al., 1992).

In this study, our aim was first to investigate a possible relaxant effect of helicidine on guinea-pig trachea, as a mechanism for its beneficial effect in airway diseases. We have then evaluated the role of prostanoids, including prostaglandin E₂, in this relaxation, and the role of epithelium as a possible source of prostanoids.

MATERIALS AND METHODS

Preparation of *Helix pomatia* Extract

The *H. pomatia* extract (1% NaCl extract) was provided by Therabel-Lucien-Pharma Company (Neuilly-sur-Seine, France). Before use, this extract was further purified as follows. In a cold room (4°C), 100 ml of crude extract (dried weight: 2.5 g) were centrifuged twice at 2000 g, and pellets (total dried weight: 377 mg) were discarded. Pooled supernatants were then processed by serial filtration in a Sartorius unit through a glass fiber prefilter, a 0.8 µm cellulose nitrate membrane, a polyester separator, and a 0.45 µm cellulose nitrate membrane, in order to eliminate any particles. The purified solution was then ultrafiltered through cellulose triacetate membranes (Sartorius tangential "easy flow" ultrafiltration, cut-off 20,000 Da) for 6 h at 4°C. The ultracentrifugation procedure was repeated twice and the fraction containing large molecular weight molecules (>20,000 Da) was collected and lyophilized.

Tissue Preparation

Tracheas were obtained from male albino Dunkin Hartley guinea-pigs (Iffa-Credo, L'Arbresle, France) weighing 250 to 300 g. Animals were anaesthetized with 60 mg/kg sodium pentobarbitone injected intraperitoneally. Tracheas were dissected out rapidly, stripped of connective tissue and opened longitudinally through the cartilage. Eight strips comprising four cartilaginous ring segments were cut from one trachea. For preparation of intact trachea, i.e., trachea with epithelium (E+), care was taken to avoid damage of the lumi-

nal surface during the entire procedure of preparing and mounting the strips. To prepare epithelium-free strips (E-), the luminal surface was rubbed gently with a cotton swab.

Measurement of Tracheal Smooth Muscle Responses

Tracheal smooth muscle contractions and relaxations were measured as changes in contractile force monitored with Narco isometric force-displacement transducers (F 60 Myograph) connected to preamplifiers and Narco recorders (Narco-Physiograph IV). Tracheal strips with (E+) or without (E-) epithelium were placed in 10 ml organ baths containing Krebs-Henseleit solution (pH 7.4) maintained at 37°C and gassed with 95% O₂ – 5% CO₂. Tissues were placed under an initial tension of 0.3 g and were washed three times at 15 min intervals. They were then equilibrated under a 2 g tension and after a 15–20 min equilibration period, experiments were initiated.

Relaxation of guinea pig tracheal smooth muscle in response to *H. pomatia* extract was measured in preparations precontracted with histamine at a concentration inducing 50% of the maximal contraction (Tschirhart et al., 1987; Frossard et al., 1989), i.e., 3 µM in E+ and 1 µM in E- tracheal strips. Cumulative concentrations of *H. pomatia* extract ranging from 0.001 to 1 mg/ml were added to the organ bath at the plateau of contraction. In experiments investigating the role of cyclooxygenase metabolites in helicidine-induced relaxation, indomethacin (2 µM) was pre-incubated for 30 min before addition of histamine.

Measurement of Eicosanoid Release

Tracheal segments (E+ or E-) corresponding to four cartilaginous ring segments and weighing 19.1 ± 0.5 mg, were placed into tubes containing 3 ml of Krebs-Henseleit solution gassed with 95% O₂ – 5% CO₂ and maintained at 37°C. After three washings at 15 min intervals and a 15 min resting period, 1 ml of incubation medium was removed to determine the basal eicosanoid production by tracheal strips. Histamine was then added at a final concentration of 3 µM or 1 µM to E+ or E- tracheal segments, respectively. Ten minutes later, 1 ml of incubation medium was sampled to determine the eicosanoid release induced by histamine. *H. pomatia* extract (0.05 or 1 mg/ml) or its vehicle was added to the remaining 1 ml. After 5 min incubation, tracheal segments were removed and the remaining fluid samples were frozen at –80°C until eicosanoid measurements. Eicosanoids, including

prostaglandin E₂ (PGE₂), thromboxane B₂ (TxB₂) as the stable metabolite of thromboxane A₂, 6-keto prostaglandin F_{1α} (6-keto PGF_{1α}) as the stable metabolite of prostaglandin I₂ and prostaglandin F_{2α} (PGF_{2α}) were measured in fluid samples by enzyme immunoassays according to the method of Pradelles et al. (1985) and using kits commercialized by Stallergenes S.A. (Fresnes, France).

Reagents and Buffers

The composition of the Krebs-Henseleit solution was (mM): NaCl, 114; KCl, 4.7; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25 and glucose, 11.7. Histamine – 2HCl and indomethacin base were purchased from Sigma Chemical Co (St. Louis, MO). Stock solutions of histamine (1 mM) were prepared in Krebs-Henseleit solution, aliquoted and stored at –20°C. Further dilutions of histamine were prepared in Krebs-Henseleit buffer. Indomethacin was prepared in 5 mM NaHCO₃ as a 2 mM stock solution, and further diluted in Krebs-Henseleit solution. Controls were preincubated with the solvent, i.e., 5 mM NaHCO₃ diluted as above. *H. pomatia* extract was dissolved and further diluted in Krebs-Henseleit buffer.

Expression of Results and Statistical Analysis

Tracheal smooth muscle responses to *H. pomatia* extract were expressed as a percentage of the tension induced by histamine. Results are means ± SEM of 6 experiments. The effects of epithelium removal and/or of indomethacin were analysed by repeated measures of analysis of variance of the entire curves.

The levels of prostaglandins and TxB₂ released from tracheal segments were expressed as pg/mg of wet weight tissue, taking into account the volume of Krebs' buffer present in the incubation tube at each step of the experiment. Results are means ± SEM of 5 experiments. Prostaglandin and TxB₂ productions before and after addition of *H. pomatia* extract were compared using a Wilcoxon rank test. A *P* value less than 5% was considered as significant.

RESULTS

Relaxation of Guinea-Pig Trachea

In E+ guinea-pig tracheal segments pre-contracted with 3 μM histamine, cumulative addition of *H. pomatia* extract (0.001–1 mg/ml) produced a dose-dependent relaxation (Fig. 1). This relaxation was immediate and

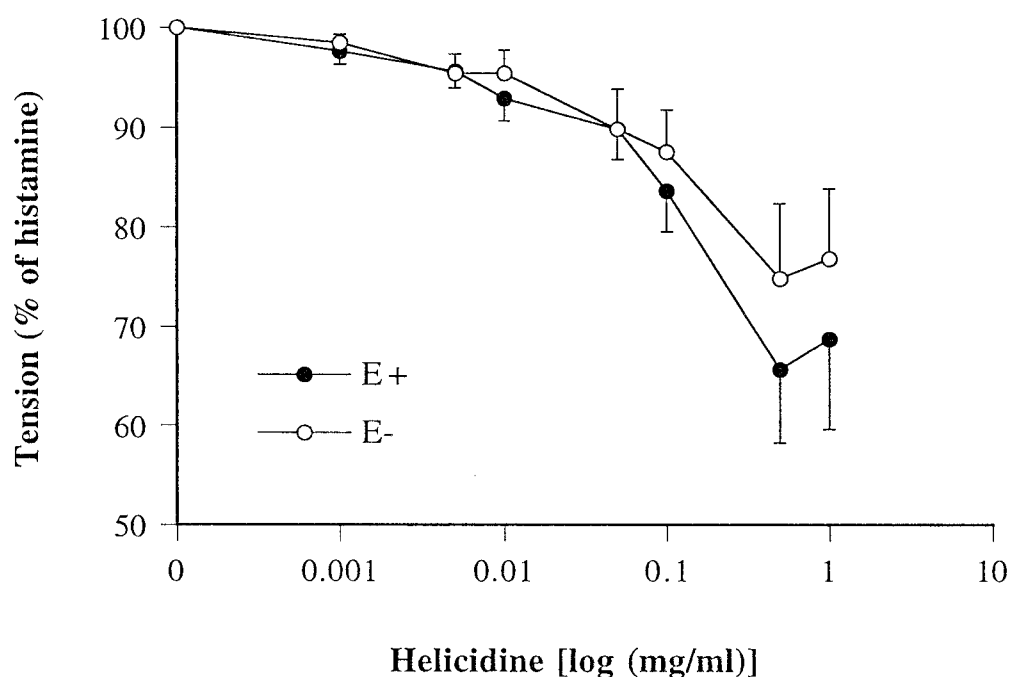


Fig. 1. Dose-dependent relaxation of intact (E+, closed circles) or epithelium-free (E-, open circles) guinea-pig tracheal segments induced by helicidine. Tracheal segments were pre-contracted with either 1 μM (E-) or 3 μM (E+) histamine before cumulative addition of 0.001 to 1 mg/l of helicidine. Results are expressed as a percentage of the tension induced by histamine and are means ± SEM of *n* = 6 experiments.

plateaued within 5 min. The threshold concentration of *H. pomatia* extract was 0.005 mg/ml and maximal relaxant effect was observed at 0.5 mg/ml *H. pomatia* extract. This maximal relaxant effect averaged $35 \pm 7\%$ of histamine-induced contraction.

In E⁻ tracheal segments pre-contracted with 1 μ M histamine, a similar dose-dependent relaxant effect of *H. pomatia* extract was observed with a threshold concentration of extract of 0.005 mg/ml and a maximal relaxant effect of $25 \pm 7.5\%$ at 0.5 mg/ml *H. pomatia*

extract (Fig. 1). No significant difference in the relaxant effect of *H. pomatia* extract was observed between tracheal segments with and without epithelium (Fig. 1).

Effect of Indomethacin on the Relaxant Effect of *Helix pomatia* Extract

In a second series of experiments, the relaxant effect of *H. pomatia* extract on E⁺ or E⁻ tracheal segments pre-contracted with histamine was studied in the presence or absence of the cyclooxygenase inhibitor, indo-

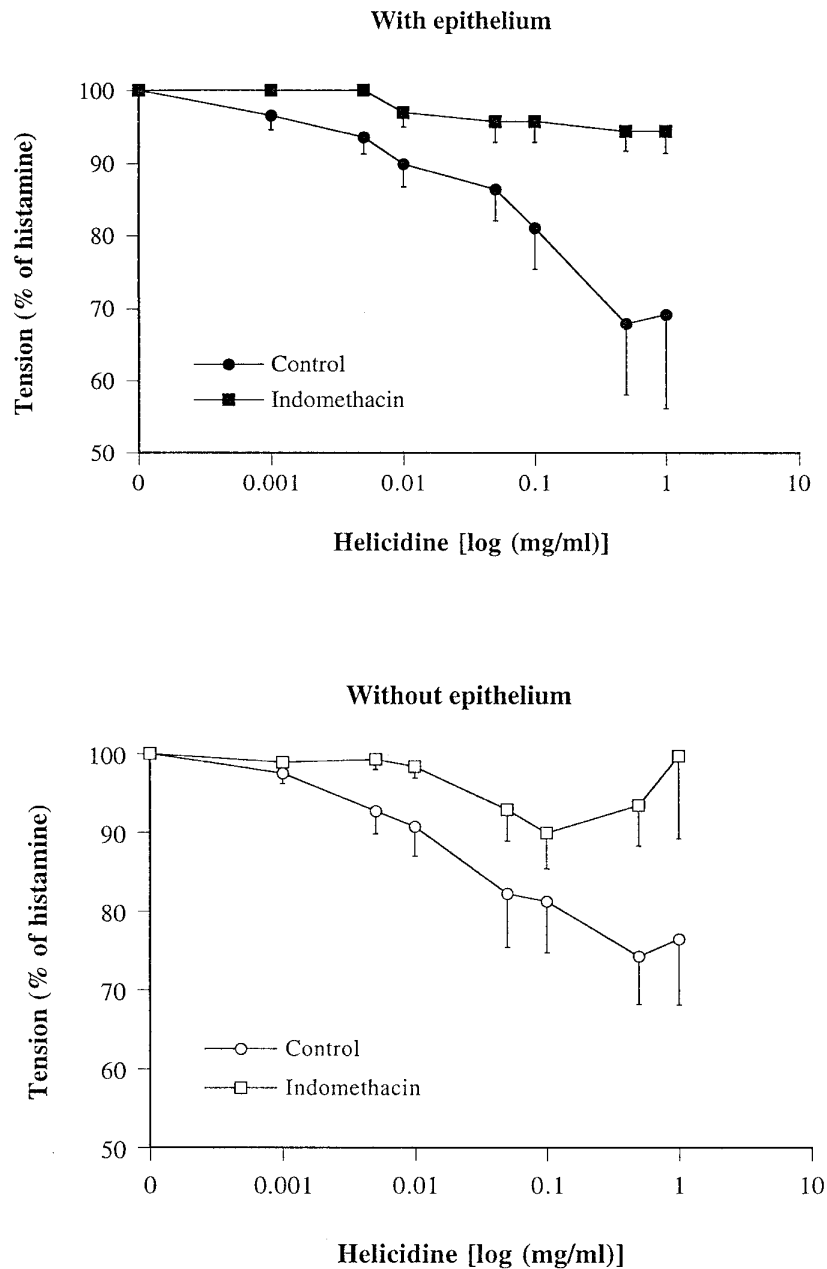


Fig. 2. Effect of indomethacin on helicidine-induced relaxation of intact (upper panel) or epithelium-free (lower panel) guinea-pig tracheal segments. Indomethacin (2 μ M, squares) was pre-incubated for 30 min prior to addition of histamine. Control strips (circles) were pretreated with the solvent alone. Contraction was induced by either 1 μ M (E⁻) or 3 μ M (E⁺) histamine before cumulative addition of 0.001 to 1 mg/ml of helicidine. Results are expressed as a percentage of the tension induced by histamine and are means \pm SEM of $n = 6$ experiments.

Table 1. Production of PGE₂, 6-keto PGF_{1α}, PGF_{2α} and TxB₂ induced by 0.05 mg/ml helicidine.

Helicidine	PGE ₂		6-keto PGF _{1α}		PGF _{2α}		TxB ₂	
	E+	E-	E+	E-	E+	E-	E+	E-
0.05 mg/ml								
Baseline	15.5 ± 1.8	11.1 ± 0.9	16.1 ± 3.7	13.3 ± 1.5	4.8 ± 0.6	4.9 ± 0.6	3.9 ± 0.7	5.87 ± 1.2
Histamine	20.6 ± 3.0	12.6 ± 1.6	35.6 ± 5.0	26.0 ± 4.1	10.4 ± 1.0	10.2 ± 1.2	31.4 ± 6.0	18.4 ± 4.3
Helicidine	20.4 ± 3.7	15.8 ± 2.0	27.6 ± 3.4	23.6 ± 2.7	10.5 ± 1.0	11.4 ± 1.0	24.7 ± 4.1	17.6 ± 3.7

Production of PGE₂, 6-keto PGF_{1α}, PGF_{2α} and TxB₂ by intact (E+) or epithelium-free (E-) guinea-pig tracheal segments at baseline, after addition of 1 μM (E-), or 3 μM (E+) histamine, and after addition of 0.05 mg/ml helicidine. Results are expressed as picograms per milligram wet weight tissue and are means ± SEM of *n* = 5 experiments.

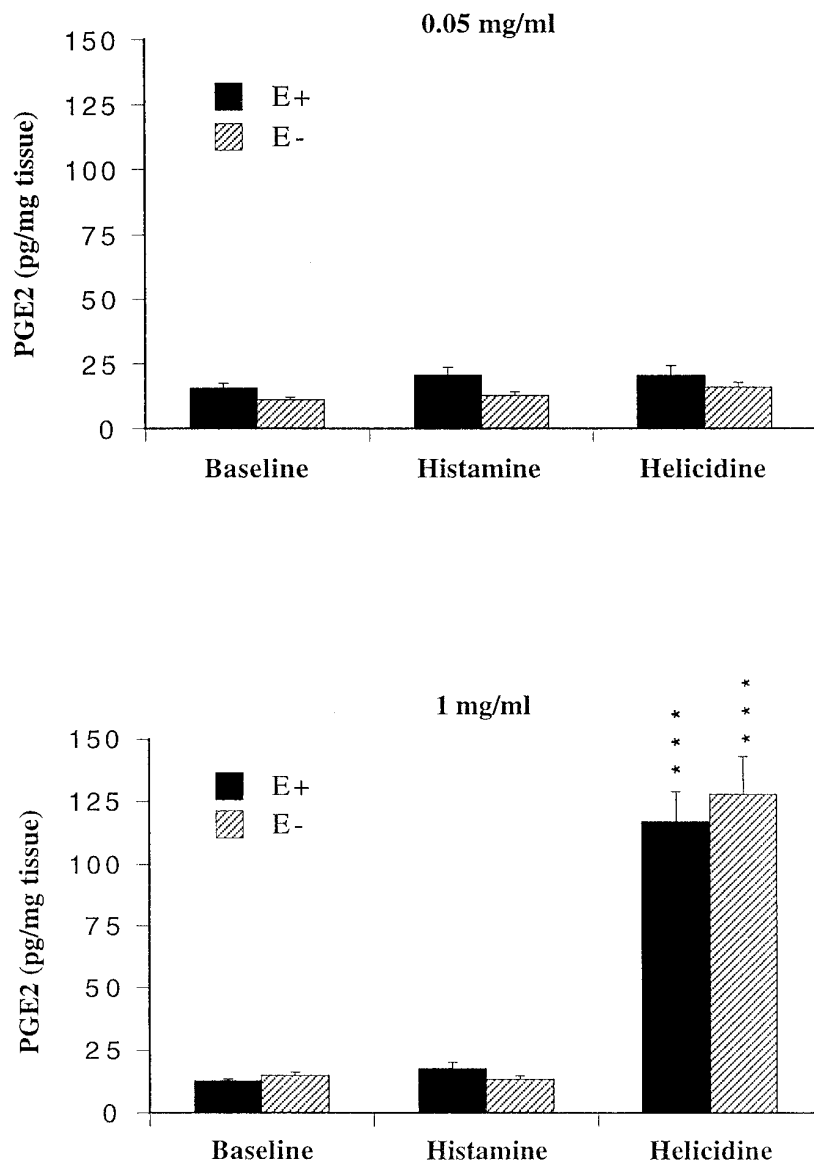


Fig. 3. PGE₂ production by intact (E+, solid bars) or epithelium-free (E-, hatched bars) guinea-pig tracheal segments at baseline, after addition of 1 μM (E-) or 3 μM (E+) histamine, and after addition of 0.05 mg/ml (upper panel) or 1 mg/ml (lower panel) of helicidine. Results are expressed as picograms per milligram wet weight tissue and are means ± SEM of *n* = 5 experiments. *** *p* < 0.01 compared to values after addition of histamine.

Table 2. Production of PGE₂, 6-keto PGF_{1α}, PGF_{2α} and TxB₂ induced by 1 mg/ml helicidine.

Helicidine	PGE ₂		6-keto PGF _{1α}		PGF _{2α}		TxB ₂	
	E+	E-	E+	E-	E+	E-	E+	E-
1 mg/ml								
Baseline	12.7 ± 0.8	14.8 ± 1.3	13.9 ± 1.6	13.7 ± 1.5	5.4 ± 0.7	5.1 ± 0.7	4.7 ± 1.7	5.0 ± 1.1
Histamine	17.6 ± 2.7	13.5 ± 1.3	38.3 ± 6.3	29.9 ± 4.5	12.1 ± 1.2	10.1 ± 1.1	32.6 ± 5.3	16.7 ± 3.6
Helicidine	116.8 ± 12.1 ^a	127.6 ± 15.3 ^a	53.0 ± 14.3	46.7 ± 9.5	16.3 ± 0.7 ^b	18.1 ± 1.5 ^b	36.6 ± 6.3	26.1 ± 4.2

Production of PGE₂, 6-keto PGF_{1α}, PGF_{2α} and TxB₂ by intact (E+) or epithelium-free (E-) guinea-pig tracheal segments at baseline, after addition of 1 μM (E-) or 3 μM (E+) histamine, and after addition of 1 mg/ml helicidine. Results are expressed as picograms per milligram wet weight tissue and are means of ± SEM. *n* = 5 experiments. ^a*P* < 0.01 and ^b*P* < 0.05 compared to values after addition of histamine using paired Student's *t* test.

methacin (2 μM). Preincubation of E+ tracheal segments with indomethacin induced near total inhibition of the dose-dependent relaxation induced by the extract (0.001–1 mg/ml) (*P* < 0.01) (Fig. 2a). A significant inhibition of the relaxant effect of the extract was also observed in E- tracheal segments pre-treated with indomethacin (*P* < 0.05) (Fig. 2b).

Eicosanoids Production by Tracheal Segments

The production of PGE₂, 6-keto PGF_{1α}, PGF_{2α} and TxB₂ by E+ or E- guinea-pig tracheal segments was assessed at baseline, after addition of histamine [1 μM (E-) or 3 μM (E+)] and after addition of 0.05 or 1 mg/ml *H. pomatia* extract. *H. pomatia* extract at the concentration of 0.05 mg/ml had no significant effect on production of either PGE₂, 6-keto PGF_{1α}, PGF_{2α} or TxB₂ in both E+ and E- tracheal segments (Table 1 and Fig. 3, upper panel). At 1 mg/ml, the extract induced a marked and significant increase in PGE₂ production over baseline and histamine-induced release in E+ and E- segments (Fig. 3, lower panel, and Table 2). Indeed, PGE₂ production increased from 17.6 ± 2.7 to 116.8 ± 12.1 pg/mg wet weight tissue, and from 13.5 ± 1.3 to 127.6 ± 15.3 pg/mg wet weight tissue, in E+ and E- tracheal segments, respectively (*n* = 5, *P* < 0.01 prior helicidine *vs* after helicidine). A slight but significant (*P* < 0.05) increase in PGF_{2α} release was also noted in E+ and E- tracheas after addition of 1 mg/ml of extract (Table 2). In contrast, no significant effect on 6-keto PGF_{1α} or TxB₂ production was noted both in E+ or E- segments (Table 2).

DISCUSSION

Our results show that a biological extract (Helicidine) prepared from the snail *H. pomatia* L. induces the relaxation of guinea-pig airway smooth muscle. The

present study describes for the first time a relaxant effect of helicidine on airway smooth muscle. These data are important as this property may, along with the sedation of cough, account for the beneficial effect of helicidine in bronchopulmonary diseases in man.

In an attempt to investigate the mechanism of the relaxant activity of *H. pomatia* extract in guinea-pig trachea, we showed that relaxation induced by this extract was similar in tracheal segments in the presence or absence of epithelium. This suggests that epithelium is not the site of action of helicidine in the airways and that epithelium does not contribute to the relaxant effect of helicidine by releasing relaxant mediators. In this respect, helicidine differs from other bronchodilators such as substance P or bradykinin, which involve epithelium activation and release of cyclooxygenase-derived relaxant prostanoids to induce airway smooth muscle relaxation (Frossard et al., 1989; Devillier et al., 1992; Schlemper et al., 1995). These data also suggest that helicidine relaxes airway smooth muscle either by acting directly on tracheal smooth muscle or by activating a intermediary cell other than the epithelial cells to release some bronchorelaxant agent.

The relaxation induced by *H. pomatia* extract in intact and epithelium-free tracheal segments was blocked by pretreatment of the tissues with the cyclooxygenase inhibitor indomethacin. Moreover, the extract induced a marked increase in the release of prostaglandin E₂ by both intact and epithelium-free tracheal segments. Taken together, these data demonstrate that helicidine-induced relaxation of guinea-pig trachea was mediated by the relaxant prostanoid prostaglandin E₂. Prostaglandin E₂ derived from epithelium has been reported to mediate the relaxant effect of substance P and bradykinin on airway smooth muscle (Frossard et al., 1989; Devillier et al., 1992; Schlemper et al., 1995). In the case of helicidine, our results indicate that prostaglandin E₂ was not derived from the tracheal epithelium. The cellular source of the

prostanoid and therefore the site of action of heliocidine in the guinea-pig trachea remain to be determined. This could involve cells of the submucosa, such as bronchial fibroblasts and the smooth muscle layer.

In conclusion, our results show that heliocidine possesses a relaxant activity on guinea-pig airway smooth muscle and that activity is mediated through the release of the relaxant prostanoid, prostaglandin E₂, from cells other than epithelial cells. In this regard, the site of action of heliocidine and the origin of prostaglandin E₂ production in the airway remain to be defined. The bronchorelaxant property of heliocidine described in this study may be involved in the beneficial effect of this drug in various pulmonary diseases in man.

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Accepted: July 14, 1997