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Inhibitory effect of CP-96,345, a non-peptide neurokinin-1-receptor antagonist, on neurogenic responses of guinea-pig isolated airway smooth muscle

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Abstract—The actions of CP-96,345, a non-peptide neurokinin-1 receptor antagonist, on the responses evoked by electrical-field stimulation or by acetylcholine and substance P in guinea-pig tracheal and bronchial muscle strips were examined. Electrical-field stimulation evoked a biphasic response, consisting of a cholinergically-mediated fast contraction followed by non-adrenergically-mediated relaxation in tracheal muscle and by a non-cholinergically-mediated slow contraction in bronchial muscle. CP-96,345 (1–10 μM) caused a concentration-dependent and non-selective inhibition in the amplitude of these neurogenic responses, where non-cholinergic contractions were more profoundly inhibited. Submaximal contractions of tracheal and bronchial muscles evoked by exogenous substance P (0·1–3 μM) were partly inhibited by CP-96,345 (1–10 μM), but acetylcholine-induced contractions were hardly inhibited. The results indicate that in guinea-pig isolated airway smooth muscle, CP-96,345 can non-selectively inhibit neurogenic responses probably via neurokinin-1 receptor-dependent and independent mechanisms.

Guinea-pig airway smooth muscle is regulated by cholinergic, adrenergic, non-adrenergic and non-cholinergic nerves, but their distributions in proximal to distal airway are different. In the tracheal muscle, excitatory cholinergic and inhibitory non-adrenergic nerves are predominant, whereas excitatory cholinergic and non-cholinergic nerves are predominant in the bronchial muscle (Coburn & Tomita 1973; Kamikawa & Shimo 1976, 1989; Andersson & Grundström 1983, 1987). Substance P or related tachykinins might function as the transmitter substance of non-cholinergic nerves (Håkanson et al 1983; Lundberg et al 1983; Leander et al 1984; Kamikawa & Shimo 1989).

Tachykinins can activate different types of tachykinin receptors: neurokinin-1 receptor for substance P, neurokinin-2 receptor for neurokinin A and neurokinin-3 receptor for neurokinin B (Frossard & Advenier 1991). None of the tachykinin antagonists previously examined possessed notable receptor selectivity and most have partial agonist activities (Håkanson et al 1983; Leander et al 1984; Kamikawa & Shimo 1989). Hence, in the present study we have investigated the effect of CP-96,345, a newly developed non-peptide tachykinin neurokinin-1 receptor antagonist (Snider et al 1991), on neurogenic responses of the guinea-pig airways.

Materials and methods

Male guinea-pigs, 300–600 g, were anaesthetized with isoflurane, bled from the cervical artery, and the tracheo-bronchial tree was excised. After cutting longitudinally at the cartilaginous portion, transverse strips, 2–3 mm wide, were excised from cervical trachea, thoracic trachea, left main bronchus and right hilus bronchus as described previously (Kamikawa & Shimo 1976, 1989, 1993). Each strip was immersed in a 10-mL organ bath filled with modified Krebs bicarbonate solution of the following composition (mM): NaCl 120, KCl 4·7, $CaCl_2$ 2·5, $MgCl_2$ 1·2, $NaHCO_3$ 25, KH_2PO_4 1·2, disodium edetate 0·03, ascorbic acid 0·12 and glucose 14 (pH 7·4). The Krebs solution always contained 20 μM choline chloride and was bubbled with 5% carbon dioxide in oxygen, and maintained at 37°C.

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The preparations were suspended under an initial tension of 0·5 g and 60 min was allowed to elapse before experiments were started. Responses of airway smooth muscle were recorded by means of an isometric transducer (Nihon Kohden TB-651T) and a Nihon Kohden polygraph (RJG-4124). Electrical-field stimulation was with rectangular pulses of 1–32 Hz frequency, 0·3 ms duration and 30 V for 20 s through bipolar platinum electrodes which were 10 mm apart and connected to a Nihon Kohden stimulator (SEN-1101). When the strip was electrically stimulated, a biphasic response was obtained at every stimulus frequency. In cervical and thoracic tracheas, the response was composed of a cholinergically-mediated fast contraction followed by a non-adrenergically-mediated relaxation, but in main and hilus bronchi a cholinergically-mediated fast contraction was followed by a non-cholinergically-mediated slow contraction (Coburn & Tomita 1973; Kamikawa & Shimo 1976, 1989; Andersson & Grundström 1983). Data are expressed as the mean ± s.e.m. Each experimental group consisted of 7–14 preparations taken from different animals. Student's *t*-test for unpaired observations was used for statistical evaluation of the data. $P < 0.05$ was considered significant.

Drugs used were CP-96,345 (dihydrochloride salt of (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)methyl)-1-azabicyclo[2.2.2]octan-3-amine, Pfizer, Groton, CT), acetylcholine chloride (Dai-ichi, Tokyo, Japan), substance P (Peptide Institute, Osaka, Japan) and isoflurane (Abbott, North Chicago, IL). To prepare the drug solutions, all drugs were dissolved in and diluted with 0·9% NaCl solution (saline). The molar concentrations of drugs in this communication refer to the final bath concentrations.

Results

Initial fast contractions to electrical-field stimulation (1–32 Hz) of tracheal and bronchial strips were unaffected by pretreatment with 1 μM CP-96,345 for 1 h, but markedly inhibited with 10 μM (Figs 1,2). Electrically-induced relaxations of cervical and thoracic tracheas were inhibited by the CP-96,345 (1–10 μM) pretreatment in a concentration-dependent manner (Fig. 1). Non-cholinergically-mediated slow contractions of main and hilar bronchi were significantly inhibited with 1 μM CP-96,345, and abolished with 10 μM (Fig. 2).

Submaximal contractions of tracheal and bronchial muscles to exogenously supplied substance P (0·1–3 μM) were partly inhibited in the presence of 1 μM CP-96,345, but 10 μM did not produce further inhibition (Figs 3,4). CP-96,345, 1 μM, did not significantly modify submaximal contractions to exogenously supplied acetylcholine (1–30 μM) but 10 μM slightly inhibited those to lower concentrations of acetylcholine (1–3 μM) (Figs 3, 4). The inhibitory actions of 10 μM CP-96,345 were not reversible by washing, and neurogenic responses or substance P-induced contractions were not fully restored to the original level even after 2 h.

Discussion

CP-96,345 is the first non-peptide antagonist for neurokinin-1 tachykinin receptors. The original report by Snider et al (1991) showed that CP-96,345 selectively and competitively antago-

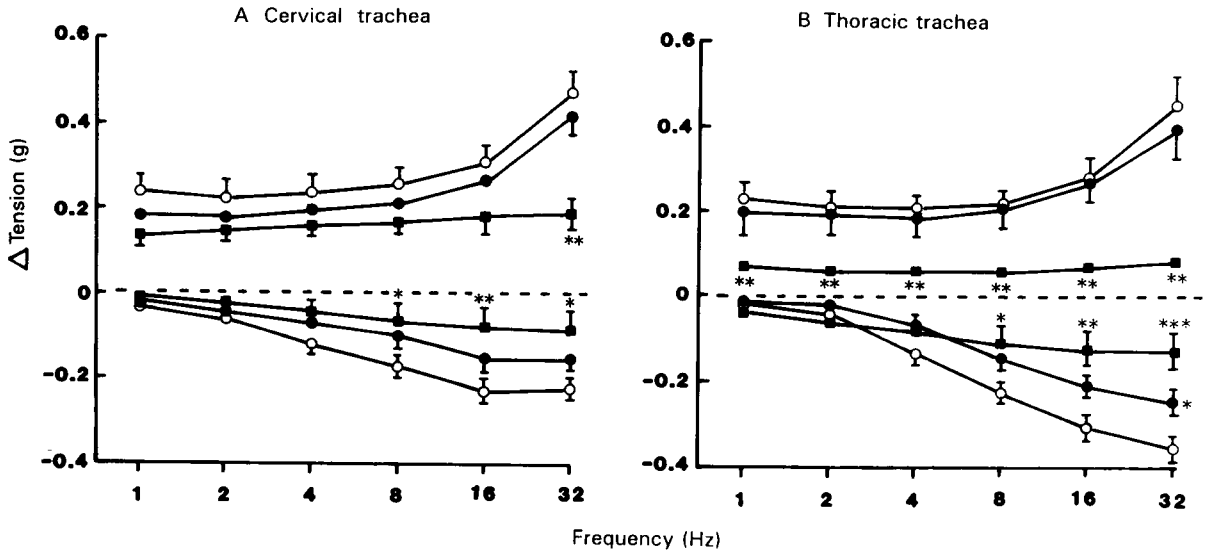

FIG. 1. Frequency-response relationship for biphasic response to electrical-field stimulation (1–32 Hz, 0·3 ms, 30 V for 20 s) of guinea-pig isolated cervical trachea (A) and thoracic trachea (B) in the absence (○, n = 13) or presence of 1 μM (●, n = 7) and 10 μM (■, n = 7) CP-96,345 for 1 h. Each point represents mean ± s.e.m. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

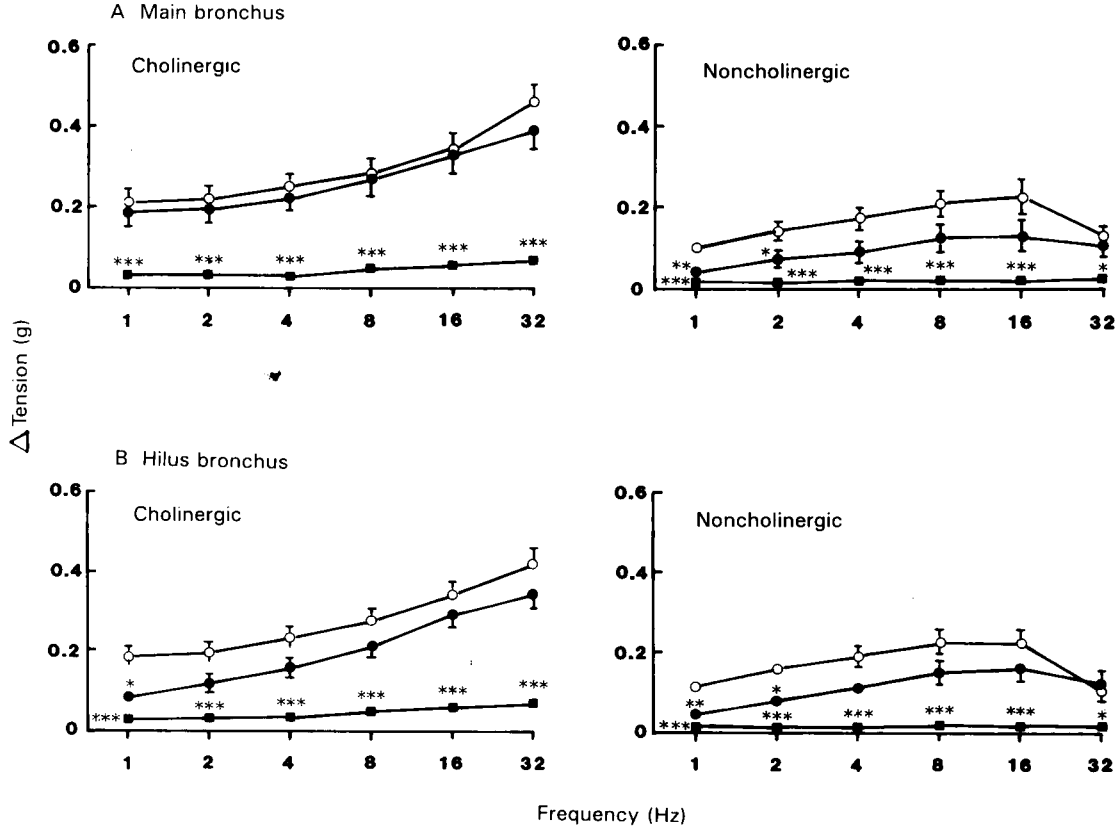

FIG. 2. Frequency-response relationship for biphasic contraction to electrical-field stimulation (1–32 Hz, 0·3 ms, 30 V for 20 s) of guinea-pig isolated main bronchus (A) and hilus bronchus (B) in the absence (○, n = 13) or presence of 1 μM (●, n = 8) and 10 μM (■, n = 7) CP-96,345 for 1 h. Left panels, cholinergically-mediated fast contraction; right panels, non-cholinergically-mediated slow contraction. Each point represents mean ± s.e.m. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

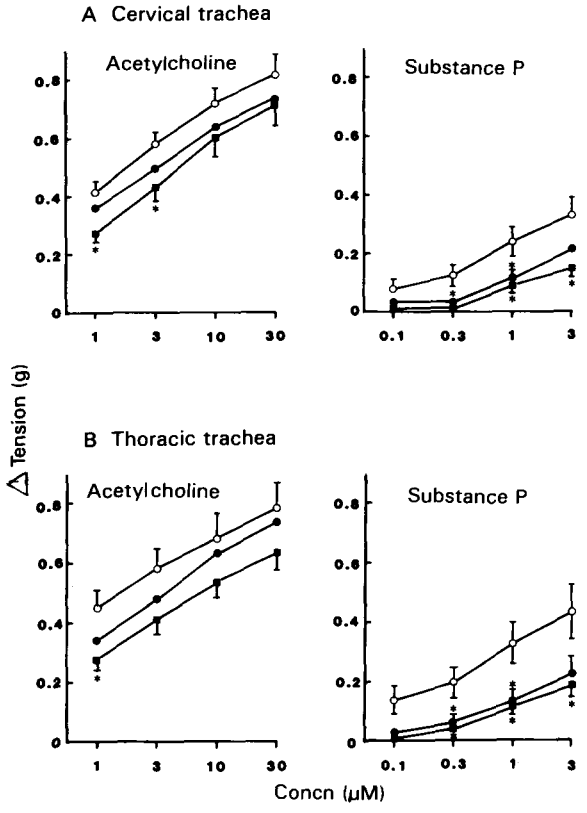

FIG. 3. Cumulative log concentration-response curves for submaximal contractions to exogenous acetylcholine and substance P of guinea-pig isolated cervical trachea (A) and thoracic trachea (B) in the absence (○, n = 13) or presence of 1 μM (●, n = 8) and 10 μM (■, n = 10) CP-96,345 for 1 h. Each point represents mean ± s.e.m. * $P < 0.05$.

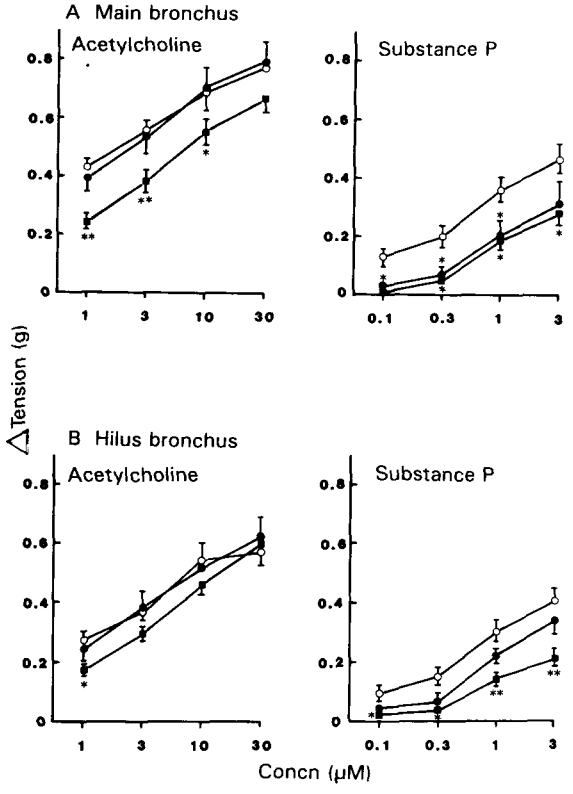

FIG. 4. Cumulative log concentration-response curves for submaximal contractions to exogenous acetylcholine and substance P of guinea-pig isolated main bronchus (A) and hilus bronchus (B) in the absence (○, n = 14) or presence of 1 μM (●, n = 8) and 10 μM (■, n = 10) CP-96,345 for 1 h. Each point represents mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.

nized the substance P-induced relaxation of dog isolated carotid artery with a pA_2 of 8·7. Also in the guinea-pig airways, it has recently been reported that CP-96,345 selectively inhibited substance P-induced bronchoconstriction (Griesbacher et al 1992). In the present experiments, substance P-induced contractions of guinea-pig isolated tracheal and bronchial muscles were partly inhibited by pretreatment with 1 μM CP-96,345, but acetylcholine induced contractions were not. The same concentration of CP-96,345 selectively inhibited the non-cholinergically-mediated slow contractions of guinea-pig bronchi without significant inhibition on the cholinergically- and non-adrenergically-mediated neurogenic responses. This indicates that some parts of non-cholinergic contraction of guinea-pig bronchi are mediated by neurokinin-1 receptors located in bronchial smooth muscles. However, 10 μM CP-96,345 non-selectively inhibited all neurogenic responses of guinea-pig airway smooth muscles, accompanied by significant inhibition of acetylcholine- and substance P-induced contractions. This indicates that higher concentrations of CP-96,345 may produce a nonspecific inhibition of airway smooth muscle reactivity, independent of neurokinin-1 receptor antagonism. Such nonspecific actions of CP-96,345 have also been reported by other investigators (Schmidt et al 1992; Nagahisa et al 1992). Previous studies showed that both neurokinin-1 and neurokinin-2 receptors mediate the non-cholinergically-mediated and tachykinin-induced contractions of guinea-pig bronchi but the relative contribution of neurokinin-2 receptors is greater than that of neurokinin-1 receptors (Frossard & Advenier 1991). Our recent paper demonstrated that SR 48968, a non-peptide neurokinin-2-receptor antagonist, selectively inhibited the non-cholinergically-mediated and neurokinin A-induced contractions of guinea-pig bronchi (Kamikawa & Shimo 1993). Taken together, a weak inhibition of non-cholinergically-mediated contraction by the low concentration of CP-96,345 observed here may suggest that neurokinin-1 receptors mediate minor parts of the non-cholinergic excitatory transmission. In contrast, more profound inhibition of neurogenic responses by higher concentrations of CP-96,345 might arise from its nonspecific action unrelated to neurokinin-1 receptor antagonism. Since neurokinin-1 receptors seem predominant in the creation of bronchial inflammation, with vasodilatation and bronchial oedema, mucosecretion, chemotaxis and activation of inflammatory cells (Frossard & Advenier 1991), the effect of CP-96,345 should be examined on the airway inflammatory reactions. Airway neurogenic inflammation might contribute to the development of bronchial hyper-responsiveness and late asthmatic response in patients with bronchial asthma (Barnes 1990; Smith 1992).

In conclusion, a non-peptide substance P antagonist, CP-96,345, can non-selectively inhibit neurally mediated responses of guinea-pig airway smooth muscles through mechanisms dependent and independent of neurokinin-1-receptor antagonism.

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Increased ex-vivo colonic generation of PAF induced by diphenylmethane stimulant laxatives in rats, mice, guinea-pigs and rabbits

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Abstract—The effects of in-vivo treatment with bisacodyl, phenolphthalein, picosulphate, sulphosuccinate, mannitol and lactulose laxatives were examined on the ex-vivo formation of platelet-activating factor (PAF) by duodenum and colon of rat, mouse, guinea-pig and rabbit. Bisacodyl (10 mg kg^{-1}), phenolphthalein (20 mg kg^{-1}) and picosulphate (10 mg kg^{-1}), but not sulphosuccinate (40 mg kg^{-1}), mannitol (50 mg kg^{-1}) or lactulose (50 mg kg^{-1}), at doses that all caused laxation, markedly increased PAF in the colon ($P < 0.01$) but not in the duodenum. Intraluminal release of acid phosphatase was also significantly increased in the colon of rats treated with bisacodyl, phenolphthalein and picosulphate, but not in colons of animals treated with sulphosuccinate, mannitol or lactulose. The data show that enhanced generation of PAF is associated with the colonic damage induced by diphenylmethane laxatives, but do not show whether this is a cause or a consequence of the pathophysiological changes.

Platelet-activating factor (PAF) is an endogenous phospholipid that produces extensive gastrointestinal hyperaemia and haemorrhage (Wallace & Whittle 1986). Administration of castor oil causes diarrhoea and intestinal damage that is associated with the increased formation of PAF along the intestine (Pinto et al 1989, 1992). In addition, observations of the intestinal action of ricinoleic acid, the intraluminal active metabolite of castor oil, have demonstrated a significant increase in PAF formation by human colonic mucosa incubated in-vitro (Capasso et al 1992). The elevated intestinal formation of PAF following castor oil was also accompanied by intraluminal release of acid phosphatase and hyperaemia suggesting a role for PAF in intestinal damage induced by castor oil. Castor oil and ricinoleic acid are commonly classified as stimulant or irritant laxatives. This group also includes phenolphthalein, bisacodyl, picosulphate, sulphosuccinate and anthraquinone-containing laxatives (Brunton 1990; Leng-Peschlow 1992). However, it has been recently demonstrated that neither senna nor the senna derivatives rhein and rhein-anthrone are able to increase intestinal PAF content (Mascolo et al 1992).

In this communication we present evidence that, like castor oil, other stimulant laxatives cause changes in the levels of PAF formed by intestinal tissue. In addition we show that some of these laxatives also stimulate intraluminal release of acid phosphatase.

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Materials and methods

Male Wistar rats, 140–150 g, were deprived of food overnight but had free access to water. In some experiments male Swiss mice, 22–25 g, male albino guinea-pigs, 250–260 g, and male New Zealand rabbits, 1·9–2 kg were also used. Laxatives (phenolphthalein 20 mg kg^{-1}, bisacodyl 10 mg kg^{-1}, sulphosuccinate 40 mg kg^{-1}, picosulphate 10 mg kg^{-1}, mannitol 50 mg kg^{-1}, lactulose 50 mg kg^{-1} (all from Sigma, Milano, Italy)) were administered intragastrically with the aid of a stomach tube and