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Tachykinins and airway microvascular leakage induced by HCl intra-oesophageal instillation

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Tachykinins and airway microvascular leakage induced by HCl intra-oesophageal instillation. S. Daoui, B. D'Agostino, L. Gallelli, X. Emonds Alt, F. Rossi, C. Advenier. ©ERS Journals Ltd 2002.

ABSTRACT: Gastro-oesophageal reflux is a common clinical disorder associated with a variety of respiratory symptoms, including chronic cough and exacerbation of asthma. In this study, the potential role of acid-induced tachykinin release was examined in guinea pigs and rabbits, by examining the effects of the tachykinin NK_1 and NK_3 receptors antagonists (SR 140333 and SR 142801, respectively) (1–10 $mg \cdot kg^{-1}$) on plasma protein extravasation induced in airways by hydrochloric acid (HCl) infusion in the oesophagus.

Guinea pigs were anaesthetised with urethane, while rabbits were subject to neuroleptoanalgesia with hypnorm. Airway vascular leakage was evaluated by measuring extravasation of Evans blue dye. All animals were pretreated with atropine (1 $mg \cdot kg^{-1}$ *i.p.*), propranolol (1 $mg \cdot kg^{-1}$ *i.p.*), phosphoramidon (2.5 $mg \cdot kg^{-1}$ *i.v.*) and saline or tachykinin receptor antagonists (1–10 $mg \cdot kg^{-1}$ *i.p.*).

Infusion of 1 N HCl into the oesophagus led to a three- and five-fold increase in plasma extravasation in the main bronchi and trachea, respectively. This increase was largely prevented by the tachykinin NK_1 and NK_3 receptor antagonists SR 140333 and SR 142801 (1–10 $mg \cdot kg^{-1}$).

These results suggest that protein extravasation in the airways, as induced by intra-oesophageal HCl infusion, is mainly dependent on the release of tachykinins, and that both NK_1 and NK_3 tachykinin receptors are involved. The results suggest that HCl-induced sensory nerve stimulation may act in the periphery on intermediate neurons and/or ganglia where NK_3 receptors have been shown to play an important role.
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Gastro-oesophageal reflux is a common clinical disorder associated with a variety of respiratory symptoms, including chronic cough and bronchoconstriction [1–4]. It has been shown to be more frequent in people with asthma [4]. Experimental studies have shown that oesophageal hydrochloric acid (HCl) perfusion induces a slight bronchoconstriction in dogs [5], cats [6] and in asthmatic patients [3, 4], and that it potentiates bronchoconstriction induced by isocapnic hyperventilation of dry air or metacholine in asthmatic subjects [4].

The mechanism by which gastro-oesophageal reflux might aggravate asthmatic symptoms remains unclear. Although instillation of acid in airways produces bronchospasm in animals [6–7], pulmonary aspiration of refluxed stomach content has not been convincingly demonstrated in clinical or scintigraphical studies of large groups of asthmatic patients with gastro-oesophageal reflux [8, 9].

Many reports have suggested that gastro-oesophageal reflux could worsen bronchoconstriction in asthmatics *via* a vagally-mediated reflex triggered by stimulation of the oesophageal acid-sensitive receptors [4, 10, 11]. However, the involvement of C-fibres and tachykinins has recently been reported in a model of airway neurogenic inflammation, induced by intra-oesophageal HCl stimulation in guinea pigs [12].

In the airways, the activation of C-fibre afferent nerves leads to local release of tachykinins, a group of neuropeptides, including substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). Tachykinins are present in several types of peripheral tissues, including gastro-intestinal tract, lung and bladder [13]. They are particularly responsible for several biological effects in the airways, including: bronchospasm, increase in microvascular permeability, vasodilatation, stimulation of glandular secretions, facilitation of cholinergic neurotransmission, and the recruitment and activation of inflammatory cells. Moreover, sensory nerves also mediate respiratory defence reflexes, such as coughing and sneezing [13–15].

The biological actions of tachykinins are mediated *via* three types of receptors, denoted tachykinin NK_1, NK_2 and NK_3, which have the highest affinity for SP, NKA and NKB, respectively [16]. This receptor classification has been established from receptor-binding and functional studies using selective agonists or antagonists for tachykinin receptors [16]. It has now been recognised that the expression of the tachykinin NK_3 receptor is confined mainly to the

central and peripheral nervous system, whilst tachykinin NK_1 and NK_2 receptors are expressed both in the nervous system and target organs, including airways [17, 18].

HAMAMOTO *et al.* [12] have shown that HCl infusion in guinea-pig oesophagus causes plasma extravasation in the airways. This effect is abolished in animals treated with capsaicin, a drug that induces a depletion of tachykinins from sensory nerves, or by the tachykinin NK_1 receptor antagonist FK 888. As these effects were observed in animals pretreated with atropine and propranolol and were abolished by bilateral vagotomy, HAMAMOTO *et al.* [12] suggested that HCl effects in that airway involve nerve pathways in the periphery.

It has been suggested that tachykinin NK_3 receptors play an important role in parasympathetic ganglia and nerve transmission in the periphery [18–20]. Thus, the current authors postulated that the tachykinin NK_3 receptor antagonist SR 142801 [21] might prevent HCl-induced neurogenic inflammation in the airways. To test this hypothesis, the airway plasma extravasation in the guinea pig was examined by stimulating the oesophagus using HCl, in the presence of SR 142801 or SR 140333, an NK_1 receptor antagonist [22]. To extend these observations to another species, a similar set of experiments were performed on rabbits.

Materials and methods

Oesophageal stimulation by hydrochloric acid

New Zealand white rabbits (2.5–2.8 kg) or tricolour guinea pigs (300–400 g) of either sex were used throughout the study. The guinea pigs were anaesthetised with urethane (1.25 $g \cdot kg^{-1}$ *i.p.*; Prolabo, Paris, France). Rabbits were subjected to neuroleptoanalgesia. They were premedicated with an intraperitoneal injection of diazepam (5 $mg \cdot mL^{-1}$, 5 $mg \cdot kg^{-1}$; Roussel Pharma SpA, Milan, Italy) and subsequently administered hypnorm (0.4 $mL \cdot kg^{-1}$; a mixture of fentanyl citrate 0.315 $mg \cdot mL^{-1}$ and fluanisone 10 $mg \cdot mL^{-1}$ *i.m.*; Janssen Pharmaceutical Ltd, Grove, Oxfordshire, UK). Guinea pig jugular veins or rabbit-ear marginal veins were cannulated for injection of drugs. Thirty min before experimentation, all animals were pretreated with atropine (1 $mg \cdot kg^{-1}$ *i.p.*; Sigma, St Louis, MO, USA) and propranolol (1 $mg \cdot kg^{-1}$ *i.p.*; Sigma) to block muscarinic and β-adrenergic receptors, and phosphoramidon (2.5 $mg \cdot kg^{-1}$ *i.v.*, 10 min before exposure to HCl (Prolabo); Sigma) to inhibit tachykinin metabolism.

The oesophageal wall was partly sectioned at the level of the 3rd–5th tracheal cartilage ring. In guinea pigs, a catheter was placed in the midoesophagus. Thus, the oesophagus was ligated at the upper portion to inhibit HCl leakage. The lower end of the oesophagus from the abdomen was then exposed and ligated to block communication between the oesophagus and the stomach. In rabbits, a second catheter with an attached latex balloon was inflated to block communication between the oesophagus and the stomach. Thus, there was no leakage of fluid from the oesophagus to the stomach.

Measurement of airway microvascular leakage

The vascular permeability was quantified by the extravasation of Evans blue dye [23]. Evans blue dye (30 $mg \cdot kg^{-1}$) was injected into the rabbit's ear marginal vein or the guinea pig's jugular vein, followed 1-min later by intra-oesophageal HCl 1 N or saline (0.4 mL). After the induction of leakage (10 min after completion of the intra-oesophageal HCl infusion), the thorax was opened and a blunt-ended 13–14-gauge needle was passed through a left ventriculotomy into the aorta. The ventricule was cross clamped and blood was expelled through an incision into the right atrium at 80 mmHg pressure with ~100 mL (guinea pig) or ~700 mL (rabbit) of phosphate buffer. The lung was then removed. The connective tissues, vasculature and parenchyma were gently scraped away and the airways were divided into four components: trachea, main bronchi and proximal and distal intrapulmonary airways [23]. The tissues were blotted dry, placed in preweighed tubes and reweighed, and their dye content was extracted in formamide at 37°C for 18 h. Dye concentration was quantified by light absorbance at 620 nm (DCP spectrophotometer; Vital, Dieren, the Netherlands), and tissue content (ng dye·mg wet weight $tissue^{-1}$) was calculated from a standard curve of dye concentrations in the 0.5–10 $\mu g \cdot mL^{-1}$ range.

Pretreatment with drugs

Guinea pigs or rabbits received a single dose (1–10 $mg \cdot kg^{-1}$ *i.p.*) of the NK_3 (SR 142801; Sanofi Recherche, Montpellier, France) or NK_1 (SR 140333; Sanofi Recherche) receptor antagonist, or vehicle 30 min before exposure to HCl.

Capsaicin pretreatment. A group of rabbits was treated with capsaicin (total dose 80 $mg \cdot kg^{-1}$ *s.c.*; Pierce, Rockford IL, USA) or vehicle (10% ethanol, 10% tween 80 and 80% NaCl 0.9%) administered over a 3-day period (5 $mg \cdot kg^{-1}$ *s.c.* on day 1, 50 $mg \cdot kg^{-1}$ *s.c.* on day 2 and 25 $mg \cdot kg^{-1}$ *s.c.* on day 3). The volume of injection was 1 $mL \cdot kg^{-1}$.

Statistical analysis of results

Data are expressed as mean±SEM. Statistical analysis of the results was performed using the unpaired t-test. p-Values of <0.05 were considered significant.

Results

Effect of phosphoramidon on airway plasma extravasation induced by oesophageal stimulation with hydrochloric acid in guinea pigs and rabbits

There were no significant differences at any airway level in extravasation of Evans blue dye after sham

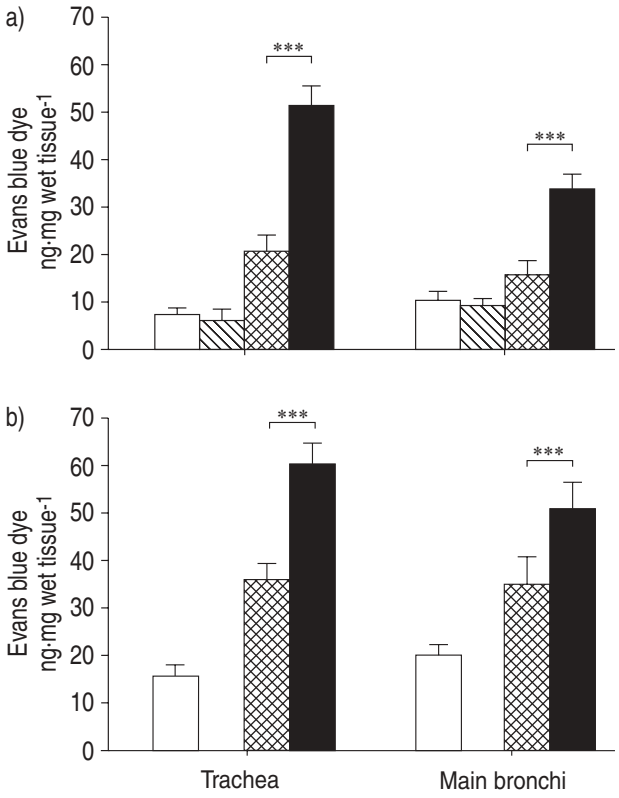

Fig. 1. – Histograms illustrating the effects of intra-oesophageal hydrochloric acid (HCl) (1 N, 10 min) on microvascular leakage in a) guinea-pig or b) rabbit airways 30 min after intraperitoneal administration of atropine and propranolol (1 mg·kg^{-1}) and 10 min after intravenous administration of phosphoramidon (2.5 mg·kg^{-1}) or saline (NaCl 0.9%). Data are presented as means± SE, n=5–6. □: saline and saline; ▨: phosphoramidon and saline; ■: saline and HCl; ■: phosphoramidon and HCl. ***: p<0.001.

stimulation with intra-oesophageal saline (0.9%) infusion in guinea pigs pretreated with either saline or phosphoramidon (fig. 1). In both species, infusion of HCl (1 N) into the oesophagus without phosphoramidon significantly increased (p<0.01) plasma extravasation in the trachea and the main bronchi, and this effect was potentiated by phosphoramidon (p<0.001) (fig. 1). No effects were observed in the proximal and distal intrapulmonary airways (results not shown).

Influence of hydrochloric acid concentration on airway plasma extravasation in guinea pigs

Only infusion of HCl at 1 N into the oesophagus in the presence of phosphoramidon induced a significant plasma extravasation in guinea pigs pretreated with atropine, propranolol and phosphoramidon, whereas 0.3 and 3 N HCl did not (fig. 2).

Effects of SR 140333 and SR 142801 on plasma extravasation in guinea-pig airways

The plasma extravasation was significantly inhibited in guinea pigs by the tachykinin receptor antagonists

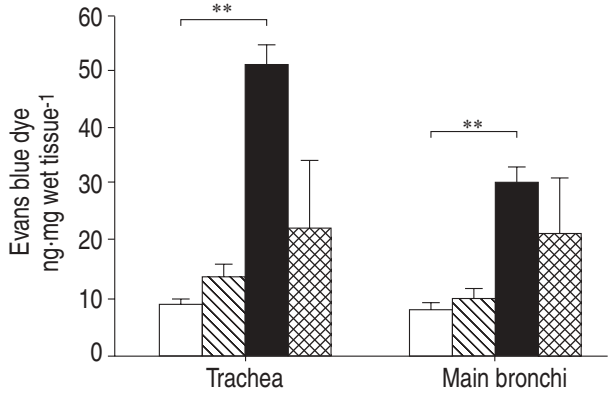

Fig. 2. – Histograms illustrating the effects of intra-oesophageal hydrochloric acid (HCl) concentration (0.3–3 N, 10 min) on airway plasma exudation in airways in guinea pigs pretreated with atropine, propranolol and phosphoramidon. Data are presented as means±SE, n=6. □: saline; ▨: HCl 0.3 N; ■: HCl 1 N; ■: HCl 3 N. **: p<0.01.

SR 140333 and SR 142801 (3–10 mg·kg^{-1}) (fig. 3). When the two tachykinin receptor antagonists were administered together (3 mg·kg^{-1} of each), the inhibition

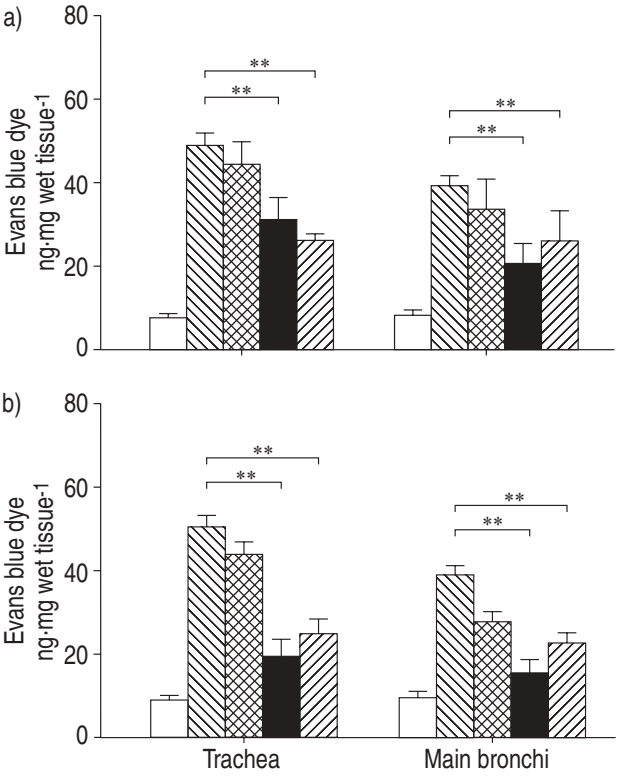

Fig. 3. – Effects of the tachykinin NK_1 or NK_3 receptor antagonists, a) SR 140333 or b) SR 142801, on the vascular leakage induced in guinea-pig trachea and main bronchi by intra-oesophageal hydrochloric acid (HCl) infusion. Tachykinin receptor antagonists (1–10 mg·kg^{-1}) were given 30 min before HCl infusion. Guinea pigs were pretreated with atropine, propranolol and phosphoramidon. Date are presented as means±SE, n=6·group^{-1}. □: control without HCl; ▨: control and HCl (1 N); ■: 1 mg·kg^{-1}; ■: 3 mg·kg^{-1}; ▨: 10 mg·kg^{-1}. **: p<0.01.

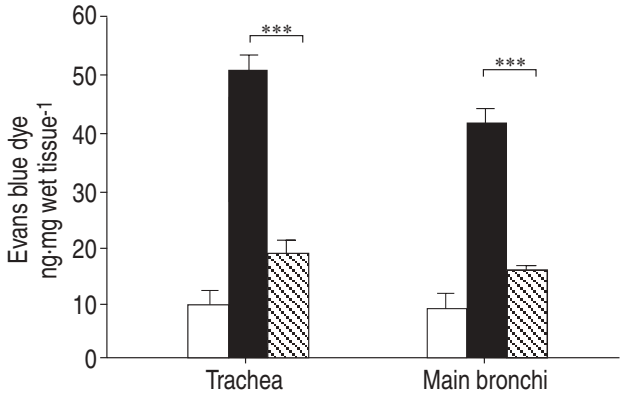

Fig. 4. – Effects of SR 140333 and SR 142801 on the vascular leakage induced by intra-oesophageal hydrochloric acid (HCl) infusion in the guinea-pig trachea and main bronchi. Doses of tachykinin receptor antagonists were given 30 min before HCl intra-oesophageal infusion. Guinea pigs were pretreated with atropine, propranolol and phosphoramidon. Data are presented as means±SE, n=6. □: control without HCl: ■: control and HCl (1 N); ▨: SR 140333 and SR 142801 (3 mg·kg^{-1} *i.p.*). ***: p<0.001.

of HCl-induced plasma extravasation in the airways was also significant (p<0.001) (fig. 4), but no additional effect or synergy was observed.

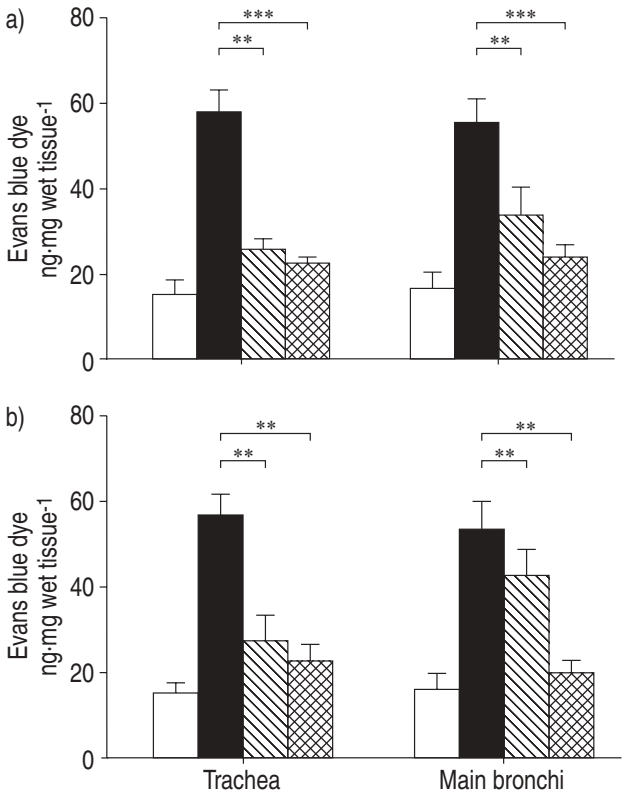

Fig. 5. – Effects of the tachykinin NK_1 or NK_3 receptor antagonists, a) SR 140333 or b) SR 14281, on the vascular leakage induced in rabbit trachea and main bronchi by intra-oesophageal hydrochloric acid (HCl) infusion. Doses of tachykinin receptor antagonists were given 30 min before intra-oesophageal HCl (1 N, 10 min) infusion. Rabbits were pretreated with atropine, propranolol and phosphoramidon. Data are presented as means±SE, n=5·group^{-1}. □: control without HCl; ■: control and HCl; ▨: 1 mg·kg^{-1}; ■: 3 mg·kg^{-1}. **: p<0.01; ***: p<0.001.

Effects of SR 140333 and SR 142801 on plasma extravasation in rabbit airways

Figure 5 shows that both SR 140333 and SR 142801 (1 mg·kg^{-1}) induced a strong inhibition (p<0.01–p<0.001) of plasma extravasation in rabbit airways following HCl instillation in the oesophagus. The effects were more pronounced in trachea than in main bronchi.

Effects of systemic capsaicin treatment on hydrochloric acid-induced airway plasma extravasation in rabbits

The plasma extravasation in airways induced by HCl was significantly abolished in the capsaicin-treated rabbits (fig. 6).

Discussion

In the present study, it has been shown that in both guinea pigs and rabbits pretreated with atropine and propranolol to block muscarinic and adrenergic receptors, oesophageal stimulation by HCl causes neurogenic inflammation in the trachea and main bronchi. Airway plasma extravasation was abolished by capsaicin in rabbits, in agreement with previous results obtained in guinea pigs by HAMAMOTO *et al.* [12]. These authors also demonstrated that this effect was inhibited in the guinea pig by the tachykinin NK_1 receptor antagonist, FK 888. The current authors provide evidence that, in addition to the tachykinin NK_1 receptor antagonist SR 140333, the NK_3 receptor antagonist SR 142801 was able to prevent neurogenic inflammation in airways both in guinea pigs and rabbits. These results suggest that nonadrenergic, noncholinergic (NANC) nerves, communicating between the oesophagus and airways, are implied in this response and that, in addition to NK_1

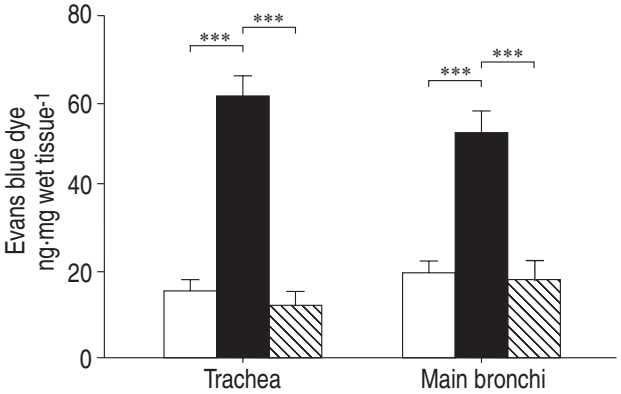

Fig. 6. – Effects of capsaicin pretreatment on plasma extravasation induced by intra-oesophageal stimulation by hydrochloric acid (HCl) (1 N, 10 min) in the rabbit. Animals were pretreated with atropine propranolol and phosphoramidon. Data are presented as means±SE, n=5. □: control without HCl; ■: control and HCl (1 N); ▨: capsaicin and HCl (1 N). ***: p<0.0 01.

receptors, NK_3 receptors are involved in transmission of the neurogenic response.

Neurogenic inflammation, induced by oesophageal instillation of HCl in the airways of guinea pigs or rabbits, is not associated with leakage from intra-oesophageal HCl [12], but involves nerve pathways communicating between the oesophagus and airways. A vagally-mediated autonomic pathway may be involved in the experimental models described here, as HAMAMOTO *et al.* [12] have shown that airway leakage induced by HCl was abolished in guinea pigs by bilateral vagotomy.

Nerve pathways communicating between the oesophagus and airway may also be in the periphery, *via* autonomic ganglia [18, 19]. Anatomical studies have demonstrated the presence of axon collaterals from visceral afferent fibres (gastro-intestinal tract, bladder, airways) in autonomic ganglia [19, 24, 25,]. Furthermore, studies carried out in isolated ganglia have demonstrated effects of these afferent nerves on the electrophysiological properties of the autonomic ganglion neurons after selective stimulation of their sensory/afferent nerve terminals [19]. Interestingly, CANNING *et al.* [20] have reported that, *in vitro*, in a preparation of guinea-pig trachea and oesophagus with associated nerves, and in the presence of propranolol, atropine, indomethacin and the tachykinin NK_2 receptor antagonist SR 48968, capsaicin elicited concentration-dependent relaxations of the trachea only when the adjacent oesophagus was intact. The effect of capsaicin was abolished by tetrodotoxin. On the basis of these results and of additional immunochemistry studies, the authors concluded that the NANC responses of the guinea pig trachea were mediated by nerve fibres emanating from parasympathetic ganglia that are intrinsic to the adjacent oesophagus [20, 26, 27]. In a similar manner, the current authors suggest that stimulation by HCl of capsaicin-sensitive fibres in the oesophagus may elicit neurogenic inflammation in airways through intermediate neurons, including parasympathetic ganglion neurons.

The results from the current study demonstrate that, in rabbits as in guinea pigs, both tachykinin NK_1 and NK_3 receptors antagonists SR 140333 and SR 142801 were able to inhibit airway extravasation induced by HCl. The inhibitory effect of NK_1 receptor antagonists has previously been shown by HAMAMOTO *et al.* [12] in guinea pigs with the compound FK 888. Inhibition by FK 888 or SR 140333 may be caused by direct inhibition of endogenous tachykinin effects on vascular cells, since NK_1 receptors play a predominant role in microvascular leakage induced by tachykinins [23, 28], although a role in nerve transmission cannot be excluded.

In contrast, the inhibitory effect of the tachykinin NK_3 receptor antagonist, SR 142801, was less anticipated, since the NK_3 receptor does not seem to be involved in the impairment of vessels and endothelial cells leading to microvascular leakage [17]. An effect of SR 142801 on NK_1 receptors can also be excluded, since the selectivity of this compound has clearly been demonstrated by radioligand binding and well characterised *in vitro* by functional assays for tachykinin receptors [21, 29, 30]. *In vivo*, the selectivity of SR 142801 is clearly suggested by two assays: in contrast to SR 48968, SR 142801 ($1\ mg{\cdot}kg^{-1}$) did not inhibit bronchoconstriction induced by [Nle^{10}] neurokinin A [4–10] in anaesthetised guinea-pigs [29]; and unlike SR 140333, SR 142801 ($1\ mg{\cdot}kg^{-1}$) failed to inhibit the hypotension induced by [Sar^9, Met $(O_2)^{11}$] substance P in guinea pigs and dogs [22, 31]. It has also been shown that SR 142801 failed to inhibit tachykinin and capsaicin-induced plasma extravasation in the ear skin of mice, an effect that could be suppressed by SR 140333 [32].

These results are in agreement with those of CANNING *et al.* [20] who demonstrated in their preparations of guinea pig trachea and oesophagus with associated nerves, that SR 142801 was able to inhibit tracheal responses to capsaicin. This suggests that, in the current *in vivo* model, HCl-induced sensory nerve stimulation may act on intermediate neurons and parasympathetic neurons where NK_3 receptors play a predominant role [18, 19].

An effect of the NK_3 receptor antagonist SR 142801 on nonadrenergic, noncholinergic nerves in periphery has also been reported in the guinea pig in other experimental models of airway hyperresponsiveness. Indeed, SR 142801 is able to prevent airway hyperresponsiveness to methacholine and the increased response to histamine on microvascular leakage in airways of guinea pigs exposed 24 h earlier to an aerosol of substance P or of citric acid [29, 33]. In this paper, it has been shown that SR 142801 has additional potential in inhibiting airway responses linked to acidity and gastro-oesophageal reflux.

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