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# Role of substance P and tachykinin receptor antagonists in citric acid-induced cough in pigs

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**Abstract**

The purpose of this work was to investigate the role of tachykinins in cough induced by citric acid (0.8 M) in pigs. With this object, we have studied the effect of citric acid on substance P content in the tracheo-bronchial tree and the effects of substance P and of tachykinin receptor antagonists on citric acid-induced cough. Citric acid exposure significantly increased substance P concentration in both broncho-alveolar and tracheal lavage fluids, while it decreased significantly the substance P content in tracheal mucosa. Substance P did not elicit cough, but significantly potentiated the citric acid-induced cough frequency. Tachykinin $NK_1$, $NK_2$ or $NK_3$ receptor antagonists, SR 140333 (nolpitantium), SR 48968 (saredutant) and SR 142801 (osanetant), respectively, significantly inhibited citric acid-induced cough. The same inhibitory effect of tachykinin receptor antagonists was observed, when substance P was nebulised before citric acid challenge. We conclude that citric acid induces in pigs a release of substance P in the tracheo-bronchial tree, which plays a sensitising role on the cough reflex. The involvement of tachykinin $NK_1$, $NK_2$, $NK_3$ receptors are also demonstrated in this reflex.


*Keywords:* Cough; Substance P; C-fibre; Tachykinin; Citric acid

## 1. Introduction

Tachykinins, especially substance P and neurokinin A, are released from non-myelinated C-fibre endings and play an important role in the physiopathology of airway disease. Substance P and neurokinin A have been shown to participate in the regulation of airway smooth muscle tone, airway mucus secretion, plasma protein extravasation, cell migration and facilitation of cholinergic neurotransmission (Barnes et al., 1991; Maggi, 1993; Maggi et al., 1993a; Ellis and Undem, 1994; Lundberg, 1996; Advenier et al., 1997; Lagente and Advenier, 1998). A role for the sensory neuropeptide system has also been proposed in cough (Widdicombe, 1995; Advenier and Emonds-Alt, 1996; Karlsson and Fuller, 1999).

Cough reflex is a symptom frequently associated with respiratory disease in pigs (Morris et al., 1995). Beyond its clinical relevancy, cough also plays a major protective role against aerial contamination of the respiratory tract. Moreover, since aerial pollutants can elicit cough, it can be considered as a good biomarker of air pollution in piggeries. However, the mechanism involved in this reflex in pigs is not yet known. Cough reflex is usually considered to be mediated in other species by irritant receptors with myelinated afferents especially rapidly adapting stretch receptors, but it has recently been found that C-fibre endings may also be involved in cough reflex (Karlsson et al., 1988). Thus, inhaled capsaicin, predominantly a C-fibre ending stimulant, causes cough in man and guinea pig (Collier and Fuller, 1984; Forsberg and Karlsson, 1986; Laude et al., 1993). Inhalation of a citric acid aqueous

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solution is known to cause cough in guinea-pig and man, and this response seems to involve sensory mechanisms, since it is inhibited in the guinea-pig by pretreatment with high doses of capsaicin, which causes degeneration of sensory nerves (Forsberg et al., 1988; Karlsson et al., 1988). Involvement of sensory neuropeptides in reflex cough has been suggested by Kohrogi et al. (1988), Takahama et al. (1993) and Ujiie et al. (1993), who showed, respectively, that substance P, neurokinin A and phosphoramidon induced cough in the guinea-pig. However, other controversial data have been obtained about the influence of aerosolised substance P, which induced no direct effect, even at high concentrations in guinea-pigs (Takahama et al., 1993; Fox et al., 1996). Moreover, species differences have been identified and a direct tussigenic effect of substance P or neurokinin A has not yet been demonstrated in humans (Joos et al., 1987). Indeed, in humans, substance P aerosols given to healthy subjects or to patients with asthma did not cause cough, but evoked a sensation of tightness in the chest of asthmatic, possibly secondary to bronchoconstriction, indicating that some sensory nerves were being stimulated (Joos et al., 1987). Cough was only reported in patients with upper airway infection upon the action of substance P but not in healthy subjects (Katsumata et al., 1989).

Finally, the view that tachykinins are involved in cough is also supported by the observation that tachykinin receptor antagonists block cough in several experimental conditions. The antitussive effect of tachykinin $NK_2$ receptor antagonists has been clearly demonstrated (Advenier et al., 1993; Robineau et al., 1994; Girard et al., 1995; Yasumitsu et al., 1996; Xiang et al., 1998) but the effect of tachykinin $NK_1$ receptor antagonist is still debated (Girard et al., 1995; Fox et al., 1996). A preventive effect of SR 142801, an antagonist of tachykinin $NK_3$ receptor, against citric acid-induced cough, has finally been reported in guinea-pigs (Daoui et al., 1998). In pigs, the effects of tachykinin receptor antagonists on the cough reflex have never been investigated so far. However, the effects of these antagonists have been tested on the contraction of ureteric smooth muscle, endocrine and exocrine pancreatic functions, intestinal motility and emetic reflex (Grélot et al., 1998; Jerde et al., 1999; Schmidt et al., 2000).

Due to conflicting results related to the investigated species and to the tachykinin receptors involved in cough, the aim of this paper was to investigate in pigs (1) the effects of citric acid on substance P content in the tracheobronchial tree; (2) the ability of substance P to induce cough and the effect of substance P on citric acid-induced cough and (3) the effect of three tachykinin receptors antagonists, SR 140333 (nolpitantium), SR 48968 (saredutant) and SR 142801 (osanetant) acting specifically on tachykinin $NK_1$, $NK_2$ and $NK_3$ receptors, respectively, on citric acid-induced cough. We also studied the effect of phosphoramidon, an inhibitor of the neutral endopeptidase on citric-induced cough in pigs.

## 2. Materials and methods

### 2.1. Animals

Healthy Belgian Landrace piglets of both sexes, weighing $12.5 \pm 1.5$ kg, were selected and subjected to experimental protocols approved by the local ethics committee. Before any experimentation, animals were introduced into an isolation room with minimal air pollution for a 10-day observation period. The mean ammonia concentration remained below 2 ppm. This was achieved by placing animals on a grid 40 cm above the floor, adding no litter, and removing the manure once daily. Animals were fed with pellets (Baby Starter, Schyns, Battice, Belgium) and received water ad libitum.

### 2.2. Cough induction test

For the cough induction test, piglets were placed individually in an inhalation chamber previously described by Urbain et al. (1996). Briefly, the closed stainless steel and plastic chamber (1.9 $m^3$) was ventilated with outside air (flow rate 10 $m^3/h$). Piglets were set on a grating located 15 cm above the floor. There was no litter and the manure was removed once daily by cleaning the floor with water to maintain the ammonia concentration below 1 ppm. Air was filtered through a fiberglass filter (CM 295, Camfil, Bruxelles, Belgium) to remove dust and aerial bacteria. This filter kept hold 95% of particles $> 1$ μm (manufacturer's specification). To avoid the effects of stress due to introduction of the pig into the chamber, cough induction test was only performed 2 days after the introduction of the animals in the chamber.

The cough induction test for pigs has been described by Moreaux et al. (1999a). Briefly, 0.8 M citric acid saline (NaCl: 0.9%) was nebulised into the chamber with an ultrasonic nebuliser (DeVilbiss, ultra-neb 2000, Somerset, PA, USA). The aerodynamic mass diameter of the droplets ranged from 0.5 to 5 μm (manufacturer's indications). The nebuliser was connected to the air entry of the inhalation chamber. The solution was delivered at a rate of 2.5 ml/min for 15 min, the ventilator of the chamber being turned off during this period. Thereafter, the inhalation chamber was ventilated with fresh air for 15 min to take away the citric acid. Coughs, distinguished from sneezes, were counted by a trained observer during these two successive 15-min periods. Sneezing was not counted because it occurred randomly. The cough frequency was expressed as the number of coughs counted over 30 min.

### 2.3. Experimental design

To study drug effects on cough frequency, the piglets were challenged twice, with a 2-day interval between the two challenges (D 1 and D 3). The values obtained during

the first cough induction test (D 1) were taken as control values. Drugs were then randomly administered intravenously (i.v.) 30 min before the second challenge. Assessed drugs were SR 140333, a tachykinin $NK_1$ receptor antagonist; SR 48968, a tachykinin $NK_2$ receptor antagonist; SR 142801, a tachykinin $NK_3$ receptor antagonist. One control group receiving no drugs except citric acid was also included in the protocol to assess the possible effect of repeated citric acid solution nebulisation. In a separated group, the animals underwent two cough induction tests (day 1 as control and day 3) and they were pretreated with the vehicle with ethanol alone before the second induction test. This vehicle did not interfere with the cough reflex induced citric acid (day 1: $138.33 \pm 11.51$ vs. day 3: $157.33 \pm 12.55$; $n = 3$).

In order to assess the possible direct coughing effect of substance P and its influence on the citric acid-induced cough, piglets were challenged twice at 2-day intervals with citric acid. Values obtained during the first test were considered as control. Just before the second test, substance P ($10^{-5}$ M) dissolved in saline solution was nebulised for 15 min. Coughs were counted during this period and the succeeding 15 min; the cough frequency was expressed as the number of coughs for the whole 30 min. Immediately after, citric acid was nebulised. In another group, animals underwent the same protocol, but before substance P challenge, phosphoramidon dissolved in saline solution was nebulised ($10^{-5}$ M) for 15 min. Coughs were counted exactly in the same way as substance P and citric acid (number of coughs/30 min). In other piglets, intravenous administrations of tachykinin receptor antagonists (SR 140333, SR48968, SR 142801) were performed 30 min before the substance P nebulisation followed by citric acid challenge to identify the type of receptors involved in the reaction.

### 2.4. Assay of substance P

In two additional groups, the animals were euthanasied with a lethal dose of thiopental (Pentothal, Abbott, Ottignies-LLN, Belgium) just after a 15-min nebulisation of 0.8 M citric acid saline solution. This was done to check the possible involvement of substance P in citric acid-induced coughing in pigs. The pigs were bled through an incision in the femoral artery, the lungs were excised, and a tracheal tube (Ruschelit number 112480, ID 4 mm) was introduced into the main bronchus of the right diaphragmatic lobe. The lobe was washed with phosphate buffer solution (PBS). The recovered fluids were pooled in a polypropylene tube containing 2 M acetic acid, to yield a final concentration of 5% by volume. The samples were kept on ice. The mean recovered amount of bronchoalveolar lavage fluid was $38 \pm 7$ ml (50.1% of the total volume of lavage fluid). The total amount of liquid used to wash the lobe was calculated taking into account the lung weight (60 ml/100 g of tissue). A section of 6-cm-long extrathoracic trachea was also excised. One end of the tracheal section was obstructed with a clamp and the tube was completely filled with PBS (mean volume: $7.5 \pm 0.75$ ml). The liquid was collected in a polypropylene tube containing pure acetic acid to yield a final concentration of 5% by volume and then kept on ice.

The pulmonary and tracheal lavage fluids were centrifuged ($800 \times g$) for 30 min at 4°C. The supernatant was separated from the pellet cells and immediately subjected to the extraction procedure. The methods used to extract substance P from lavage fluid and to determine substance P concentration have been described in detail by Nemmar et al. (1998). Briefly, the centrifuged liquids were filtered through a 0.45-μm polyester membrane (Macherey-Nagel D-52313, Düren, Germany) and concentrated with Sep Pak C18 Cartridges (Waters, Milford, MA, USA) pre-conditioned with 20 ml methanol and washed with 20 ml distilled water. The pulmonary and tracheal lavage liquids were injected into the cartridges, which were then washed with 3 ml distilled water. Then, the peptide was eluted with 8 ml aqueous solution containing 70% acetonitrile and 0.1% trifluoroacetic acid. The eluates were vacuum-dried and kept at −20°C until the assays were performed.

Immediately after death, 1-$cm^2$ strips of mucosa were sampled from the main left bronchus and the intrathoracic part of trachea, just above the right accessory bronchus. The strips were weighed, introduced into a polypropylene tube with 500 μl PBS and 5 ml of 2 M acetic acid, and mixed with an ultraturax. After incubation of the tube at 90°C for 10 min, the samples were centrifuged ($2400 \times g$) for 30 min. The supernatant was separated from the pellet cells and immediately vacuum-dried and kept at −20°C until the assays were performed. The radio-immuno assay (RIA) procedure used in this study, developed in this laboratory, has been described (Nemmar et al., 1998).

### 2.5. Drugs and dosages

Drugs and dosages used were: SR 140333 ((*S*)1-(2-(3-(3,4-dichlorophenyl)-1-(3-*iso*-propoxyphenylacetyl) piperidin-(3-yl)ethyl)-phenyl-1-azoniabicyclo (2.2.2) octane chloride) (1 mg $kg^{-1}$) (Sanofi, Montpellier, France), SR 48968 ((*S*)-*N*-methyl-*N*(4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl)benzamide) (1 mg $kg^{-1}$) (Sanofi, Montpellier, France), SR 142801 ((*R*)-(*N*)-(1-(3-(L-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-*N*-methylacetamide) (1 mg $kg^{-1}$) (Sanofi, Montpellier, France). Sterile saline was prepared for i.v. administration. All drugs were dissolved in ethanolic saline (NaCl: 0.9% and ethanol 25 vol/100). Substance P (Bachem, Paris, France) and phosphoramidon (R-7385, Sigma, Bornem, Belgium) were dissolved in saline (0.9%)

and kept on ice before the experience. Citric acid (Merck ref 1.00244, Germany) was dissolved in saline.

### 2.6. Expression of data and statistical analysis

All data are expressed as means ± S.E.M. and were subjected first for normal distribution and then for statistical significance by analysis of variance (ANOVA). The paired comparison of data obtained on day 1 and day 3 was selected due to inter-individual variability of citric acid-induced response in pigs (Moreaux et al., 1999a). When statistical significance was reached, data within a same group were compared using a paired Student's *t*-test. Owing to the rather high inter-individual variability of citric-acid-induced coughing, the control and test groups were compared using data expressed as a percentage of change between the values measured on day 1 and day 3 [((day 3 − day 1)/day 1) × 100]. An unpaired Student's *t*-test was used to compare the corresponding values. Results were considered significant when the $P$ value did not exceed 0.05.

## 3. Results

### 3.1. Effect of citric acid on substance P content in the lung

Table 1 shows that citric acid stimulation significantly increased the substance P concentration in both broncho-alveolar (+201%) and tracheal lavage fluids (+221%). The opposite tendency was observed in tracheal and bronchial tissues but the differences were significant only in tracheal mucosa (−55%).

### 3.2. Effect of substance P on cough and on citric acid-induced cough reflex

Citric acid-induced cough on day 1 and day 3 in the control group but no significant differences were observed between the data obtained during these two challenges [day 1: 192 ± 39 vs. day 3: 172 ± 37 cough/30 min ($n = 5$); percentage of inhibition: −6 ± 11%]. Fig. 1 shows that substance P alone when nebulised at a concentration of $10^{-5}$ M for 15 min, just before a provocation test with citric acid, did not elicit cough but significantly enhanced the response to citric acid (148 ± 51%) (Fig. 1A) and this effect was similar after a pre-treatment with phosphoramidon administered before substance P challenge following citric acid exposure (103 ± 26%) (Fig. 1B). When compared to the data recorded in the control group, these percentages were significantly higher ($P < 0.05$ and $P < 0.01$, respectively).

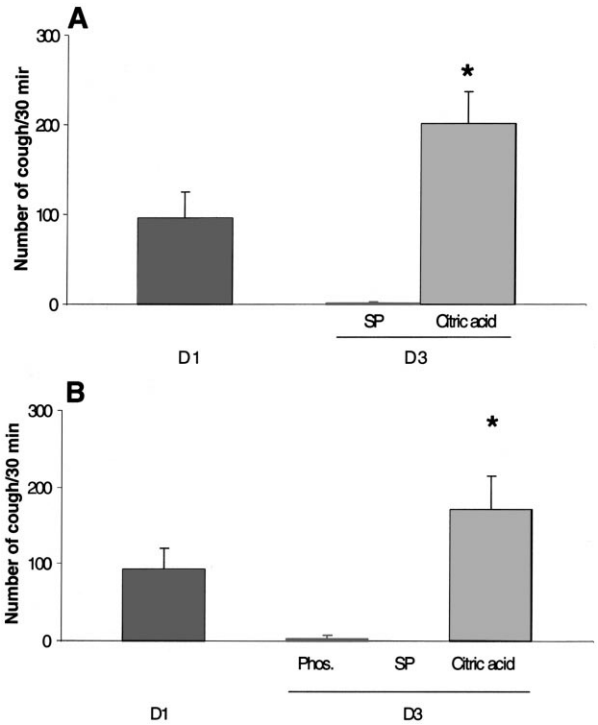


Fig. 1. Effects of a substance P and phosphoramidon challenge on spontaneous cough and on citric acid-induced cough frequency. Substance P was nebulised at the rate of $10^{-5}$ M for 15 min just before citric acid inhalation ($n = 4$) (A). The same protocol was repeated but phosphoramidon ($10^{-5}$ M) was nebulised for 15 min just before substance P administration ($n = 4$) (B). Provocation tests were performed 2 days apart (D1, D3), first day is control value. The values measured on day 3 were compared to those obtained on day 1 (control value). Data are expressed as means ± S.E.M. * Indicates a significant difference between the values measured on day 1 and day 3 ($P < 0.05$).

Table 1
Effect of 0.8 M citric acid solution nebulisation on substance P contents in broncho-alveolar and tracheal lavage fluids (BALF; TLF) and in mucosa strips sampled in the trachea and main left bronchus

| | BALF (pg/10 ml) | TLF (pg/10 ml) | Tracheal mucosa (pg/100 mg mucosa) | Airway mucosa (pg/100 mg mucosa) |
|---|---|---|---|---|
| Control group | 21.22 ± 9.45 ($n = 4$) | 43.75 ± 9.85 ($n = 4$) | 189.60 ± 44.22 ($n = 4$) | 189.32 ± 70.18 ($n = 4$) |
| Citric acid | 63.81 ± 16.15[a] ($n = 4$) | 140.61 ± 52.15[a] ($n = 4$) | 85.79 ± 16.35[a] ($n = 4$) | 88.93 ± 33.85 ($n = 4$) |

Substance P was extracted from the liquids and mucosa and titrated by a RIA procedure. Values are expressed as means ± S.E.M.
[a] Indicates a significant difference between control and test values ($P < 0.05$).

### 3.3. Effects of tachykinin receptor antagonists on citric acid-induced cough

The blockade of tachykinin $NK_1$, $NK_2$ or $NK_3$ receptors with SR 140333, SR 48968 and SR 142801, respectively, significantly reduced the cough frequency compared to that measured on day 1 (Fig. 2A). The mean percentages of inhibition caused by SR 140333 ($-46 \pm 13\%$), SR 48968 ($-59 \pm 9\%$) and SR 142801 ($-54 \pm 6\%$) were significantly greater than the negative changes recorded in the control group ($-6 \pm 11\%$) ($P < 0.01$).

When animals were exposed to substance P before citric acid challenge, the three tachykinin $NK_1$, $NK_2$, $NK_3$ receptor antagonists, SR 140333, SR 48968 and SR 142801, respectively, significantly blocked the response to citric acid (Fig. 2B). The more marked effect was obtained with SR 142801, which induced a $90 \pm 4\%$ inhibition of cough frequency. The percentage of inhibition recorded after SR 140333 and SR 48968 administration were $-60 \pm 13\%$ and $-58 \pm 15\%$, respectively. When these three percentages of inhibition were compared with the percentage recorded in control group ($-6 \pm 11\%$), a significant difference was observed ($P < 0.01$).

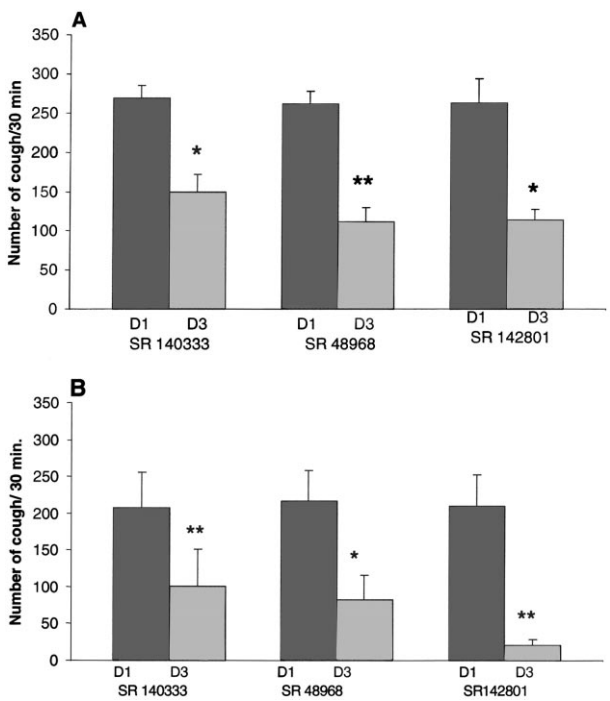


Fig. 2. Effects of SR 140333, a tachykinin $NK_1$ receptor antagonist (1 mg $kg^{-1}$, IV, $n = 5$); SR 48968, a tachykinin $NK_2$ receptor antagonist (1 mg $kg^{-1}$, IV, $n = 5$) and SR 142801, a tachykinin $NK_3$ receptor antagonist (1 mg $kg^{-1}$, IV, $n = 4$) on citric acid-induced cough in unanaesthetised pigs (A). The influence of these antagonists on the responses induced by citric acid nebulisation with a prior challenge of substance P ($10^{-5}$ M) was also investigated ($n = 4$) (B). Provocation tests were performed 2 days apart (D1, D3), the first day is the control value. The animals were pretreated with drugs 30 min before the second test. The values obtained on day 3 were compared to those obtained on day 1. Data are expressed as means ± S.E.M. * Indicates a significant difference between the values measured on day 1 and day 3 ($^{*}P < 0.05$; $^{**}P < 0.01$).

## 4. Discussion

The present study shows that citric acid inhalation in pigs can elicit the release of substance P from C-fibres in the tracheo-bronchial tree, and that this tachykinin potentiates the citric acid-induced cough reflex acting through tachykinin $NK_1$, $NK_2$ and $NK_3$ receptors.

In a previous work (Moreaux et al., 1999a), it has been demonstrated that citric acid nebulisation elicits cough reflex in pigs as it does in guinea-pigs and human (Forsberg et al., 1988; Laude et al., 1993; Daoui et al., 1998). It appears that inter-individual variability in this response can be rather high compared to the reported data in other species. Since the response to citric acid administration repeated two times at a 2-day interval was similar, this protocol was selected in this study to investigate the influence of drugs on citric acid challenge; the data obtained during the second test were compared to those recorded during the first one, considered as control.

During citric acid nebulisation, it has been demonstrated that bronchoconstriction can occur simultaneously with cough reflex. While bronchoconstriction can contribute to the activation of the cough reflex, the two phenomena do not seem to be linked. Indeed, in guinea-pigs, salbutamol can inhibit cough induced by citric acid but at higher doses than that inhibiting bronchoconstriction (Girard et al., 1995). In pigs, the cough reflex elicited by citric acid is not influenced by a pre-treatment with theophylline (8 and 15 mg/kg, i.v.) (Moreaux et al., 1999b) and no change in enhanced pause (Penh) considered as a bronchoconstriction index as measured by whole body plethysmography was observed (unpublished data). These two arguments allow excluding the contribution of bronchoconstriction in the cough reflex in pigs.

Capsaicin, a pungent extract of peppers, remains the reference drug to induce tachykinins release from C-fibres due to its specific irritant properties at this level through capsaicin-sensitive receptors (Saria et al., 1988; Lou et al., 1991). Many other substances have been suggested to activate C-fibres, but most of experimental evidences supporting this mechanism are based on the inhibitory effects of tachykinin receptor antagonists. The release of tachykinins upon the action of these stimuli remains scarcely documented. Using different experimental model, it has been demonstrated than substance P can be released upon the action of some chemical compounds as methacholine, histamine, capsaicin and carbachol (Saria et al., 1988; Manzini et al., 1989; Martins et al., 1991a,b; Nemmar et al., 1999a,b). In human subjects and guinea-pigs, hyper-responsiveness and cough induction by ozone have also been related with a local substance P release (Murlas et al., 1992; Hazbun et al., 1993). Citric acid is thought to act partially on C-fibre to elicit cough. Our data now

demonstrate that inhalation of citric acid in pigs elicits the release of substance P in airways and induces simultaneously cough giving a strong argument in favour of a peripheral role for substance P in the cough reflex. The decrease in the substance P content in the tracheal mucosa and the increase in the broncho-alveolar and tracheal lavage fluids as recorded in our pigs are likely due to a release of this peptide from C-fibres combined with the enzymatic degradation occurring at this level (Hazbun et al., 1993; Nadel, 1994; Sekizawa et al., 1995, 1996; Fox, 1996).

The fact that substance P release and cough occur simultaneously in our model after citric acid aerosol is not sufficient to demonstrate the role of this peptide in the reflex. The direct effect of substance P on cough reflex needs to be investigated. Few studies about the effect of substance P aerosols are available. Kohrogi et al. (1988) have showed that substance P can induce a cough reflex in guinea-pigs at very low concentrations ($10^{-18}$–$10^{-14}$ M) but these data have never been confirmed so far. Takahama et al. (1993) and Fox et al. (1996) have showed that substance P given at concentrations up to $10^{-4}$ M did not evoke cough in guinea-pigs. Similar data were obtained in healthy human and asthmatic patients who do not respond to substance P inhalation (from $10^{-6}$ to $10^{-3}$ M) (Joos et al., 1987). Only the patients with airway diseases such as upper airways infections react to substance P challenge ($10^{-15}$ M) by a cough response (Katsumata et al., 1989). Substance P does not elicit cough in pigs confirming the data previously reported in guinea-pigs, healthy human volunteers and asthmatic patients but enhances the response to citric acid. This sensitising role was also reported by Fox et al. (1996) in guinea-pigs but was not related to a direct activation of airway sensory nerves (Fox, 1996; Fox et al., 1996). The mechanisms explaining the sensitising role of substance P on the cough reflex remain unclear.

The degradation of substance P by the neutral endopeptidase in airway epithelial cells could limit the response elicited by an aerosol challenge. This enzymatic activity explains why phosphoramidon, an inhibitor of neutral endopeptidase, has been reported to induce cough in guinea-pigs (Takahama et al., 1995). To check this hypothesis, pigs were pretreated with phosphoramidon but the potentiating effect of substance P vs. cough induced by citric acid was not modified as previously reported by Fox et al. (1996) in guinea-pigs pretreated with phosphoramidon and captopril. Thus, a decrease in bioavailability of aerosolised substance P does not seem to explain the absence of cough after a substance P challenge in both species. The fact that a high concentration of substance P ($10^{-5}$ M) was used, possibly eliciting a maximum sensitising effect, could explain our observations. A second hypothesis could be that the neutral endopeptidase activity is low in pigs explaining why high concentrations of substance P are detected in broncho-alveolar and tracheal lavage fluids after citric acid challenge.

The role of the substance P in the cough reflex in pigs is confirmed by the inhibiting effect of neurokinin receptors antagonists. The use of specific tachykinin $NK_1$, $NK_2$ and $NK_3$ receptor antagonists allowed to identify the involved subtypes. The doses used in this species are those used in other species, especially in guinea-pig (Girard et al., 1995; Yasumitsu et al., 1996; Bolser et al., 1997; Daoui et al., 1998). All these drugs have shown a very high selectivity for these corresponding receptors (Advenier et al., 1992; Maggi et al., 1993b; Jensen et al., 1994; Nguyen et al., 1996; Beaujouan et al., 1997) and their selectivity has been demonstrated in different tissues from several animal species (Advenier et al., 1992; Maggi et al., 1993b; Nguyen et al., 1996; Beaujouan et al., 1997). The antagonists used in the present study have never been investigated in vivo in pigs. In vitro, they have been tested on the contraction of ureteric smooth muscle, endocrine and exocrine pancreatic functions, intestinal motility and emetic reflex (Grélot et al., 1998; Jerde et al., 1999; Schmidt et al., 2000). However, no binding data or comparison of potency are available. The inhibition of citric acid-induced cough reflex in pigs by SR 48968, a tachykinin $NK_2$ receptor antagonist, confirms the role played by tachykinin $NK_2$ receptors as previously identified in guinea-pigs, cats and human (Advenier et al., 1993; Maggi et al., 1993a; Girard et al., 1995; Bolser et al., 1997). In guinea-pigs and cats, the cough reflex is inhibited when tachykinin $NK_1$ receptors are blocked with CP-99,994 and FK 888 (Fujii et al., 1992; Yasumitsu et al., 1996; Bolser et al., 1997). In contrast, Girard et al. (1995) and Xiang et al. (1998) reported no inhibitory effect of SR 140333, another tachykinin $NK_1$ receptor antagonist, on the cough reflex in guinea-pigs. While, when SR 140333 was administered simultaneously with SR 48968, the inhibitory effect of the latter was more marked, suggesting a synergy between these tachykinin antagonists ($NK_1$ and $NK_2$) (Girard et al., 1995). Whatever it is, the role played by the tachykinin $NK_1$ receptor in pigs is clearly illustrated by our data. To explain the discrepancy between the effects induced by the different $NK_1$ antagonists in guinea-pigs, Bolser et al. (1997) proposed that SR 140333, contrary to CP-99,994, cannot penetrate the central nervous system (CNS) to centrally inhibit coughing. If this hypothesis is true, our data clearly show that SR 140333 can inhibit coughing by a peripheral mechanism in pigs. However, this pharmacokinetic difference between CP-99,994 and SR 140333 was only suspected. The involvement of different subtypes of tachykinin $NK_1$ receptors differently blocked by SR 140333 and CP-99,994 and located in the CNS and in peripheral tissues was also suspected (Bolser et al., 1997). The role of tachykinin $NK_3$ receptors in the cough reflex has been scarcely investigated so far. Daoui et al. (1998) showed the inhibitory effect of SR 142801, a tachykinin $NK_3$ receptor antagonist, on the citric acid-induced cough reflex in guinea-pigs. By blocking these receptors, SR 142801 can also prevent

airway hyper-responsiveness to acetylcholine and histamine-induced increase in microvascular permeability in guinea-pigs previously exposed to aerosolised citric acid and can reduce the bronchoconstriction when tachykinin $NK_2$ receptors are blocked by SR 48968 (Daoui et al., 1998). For the first time, the effect of tachykinin $NK_3$ receptor antagonists on the cough reflex is documented in another species than guinea-pigs. However, when substance P is nebulised before citric acid, the percentage of inhibition induced by SR 142801, a $NK_3$ tachykinin receptor antagonist, is significantly more marked than that observed when SR 142801 is administered alone before citric acid challenge. The fact that tachykinins other than substance P can be release upon the action of citric acid could explain this difference.

In conclusion, our results show that citric acid induces in the tracheo-bronchial tree a release of substance P, which plays a sensitising role on the cough reflex in pigs through the stimulation of tachykinin $NK_1$, $NK_2$, $NK_3$ receptors.

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