Substance P contributes to rapidly adapting receptor responses to pulmonary venous congestion in rabbits

A. C. Bonham, K. S. Kott, K. Ravi, C. T. Kappagoda and J. P. Joad

Departments of Internal Medicine, Pharmacology and Pediatrics, University of California, Davis, CA 95616, USA

1. This study tested the hypothesis that substance P stimulates rapidly adapting receptors (RARs), contributes to the increase in RAR activity produced by mild pulmonary congestion, and evokes an augmented response from RARs when combined with near-threshold levels of pulmonary congestion.

2. RAR activity, peak tracheal pressure, arterial blood pressure and left atrial pressure were measured in paralysed, anaesthetized and ventilated rabbits. Substance P was given I.V. in one-half log incremental doses to a maximum of 3 μg kg^{-1}. Mild pulmonary congestion was produced by inflating a balloon in the left atrium to increase left atrial pressure by 5 mmHg. Near-threshold levels of pulmonary congestion were produced by increasing left atrial pressure by 2 mmHg.

3. Substance P produced dose-dependent increases in RAR activity. The highest dose given increased the activity from 1·3 $\pm$ 0·5 to 11·0 $\pm$ 3·1 impulses bin^{-1}. Increases in left atrial pressure of 5 mmHg increased RAR activity from 3·8 $\pm$ 1·4 to 14·7 $\pm$ 3·9 impulses bin^{-1}. Blockade of NK_1 receptors with CP 96345 significantly attenuated RAR responses to substance P and to mild pulmonary congestion.

4. Doses of substance P, which alone had no effect, stimulated the RARs when delivered during near-threshold levels of pulmonary congestion.

5. The findings suggest that substance P augments the stimulatory effect of mild pulmonary congestion on RAR activity, most probably by enhancing hydraulically induced microvascular leak.

The rapidly adapting 'irritant' receptors (RARs) and the C fibre receptors in the lungs and airways elicit various components of the neural airway defense reflexes, most notably mucus secretion, bronchoconstriction and changes in ventilatory pattern (Widdicombe, 1977; Karlsson, Sant' Ambrogio & Widdicombe, 1988; Coleridge & Coleridge, 1994). The RARs are stimulated by lung hyperinflation and deflation (Widdicombe, 1954), environmental toxins including cigarette smoke (Sellick & Widdicombe, 1971; Ravi, Kappagoda & Bonham, 1994*b*) and ozone (Coleridge, Coleridge, Schelegle & Green, 1993) and by autocoids including histamine (Sellick & Widdicombe, 1971), bradykinin (Hargreaves, Ravi, Senaratne & Kappagoda, 1992; Hargreaves, Ravi & Kappagoda, 1993) and prostaglandins (Coleridge, Coleridge, Ginzel, Baker, Banzeff & Morrison, 1976). One of the fundamental mechanisms by which some stimuli excite the RARs may be the transfer of fluid into the perivascular interstitial space of the airways. Kappagoda and colleagues have demonstrated that subtle manipulations of Starling forces, which increase fluid flux into the interstitial spaces, stimulate the RARs. Such manoeuvres, which include mild levels of pulmonary venous congestion (Kappagoda, Man & Teo, 1987; Hargreaves, Ravi & Kappagoda, 1991) and obstruction of lymphatic drainage from the lungs (Hargreaves *et al.* 1991; Ravi, Bonham & Kappagoda, 1994*a*), provide a sustained and consistent stimulus to the RARs. These manipulations also stimulate the airway C fibres, but to a lesser and variable extent (Kappagoda *et al.* 1987).

Of related interest are the findings that the airway C fibres contain substance P, along with neurokinin A (NKA) and calcitonin gene related peptide (CGRP) (Maggi, Giachetti, Dey & Said, 1995). The local release of substance P from the C fibre nerve terminals evoked either by antidromic stimulation of the vagus nerve or by administration of capsaicin causes microvascular leak in the airways, an effect which is mimicked by exogenous application of substance P or its agonists and blocked by substance P (NK_1) receptor antagonists (Eglezos, Giuliani, Viti & Maggi, 1991; Lei, Barnes & Rogers, 1992; Lembeck, Donnerer, Tsuchiya & Nagahisa, 1992; Yiamouyiannis, Stengel, Cockerham & Silbaugh, 1995; Maggi *et al.* 1995). Thus, exogenous administration of substance P and conditions associated with its endogenous release would be

expected to stimulate the RARs, at least in part, by increasing microvascular permeability. Given that mild pulmonary congestion provides a modest stimulus to the airway C fibres, the subsequent release of small amounts of substance P from these fibres under such conditions may be sufficient to amplify the hydraulically induced increase in fluid flux and thus provide a more robust stimulus to the RARs. Accordingly, this study was designed to test the hypotheses that: (1) substance P stimulates the RARs via an NK_1 receptor mechanism; (2) substance P contributes to the increase in RAR activity produced by mild pulmonary venous congestion, and thus NK_1 receptor antagonism attenuates the increase; and (3) substance P-induced increases in RAR activity are greater during near-threshold levels of pulmonary venous congestion.

METHODS

General surgical preparation

New Zealand White male rabbits (body weight, 2·9–3·9 kg) were pre-anaesthetized with ketamine HCl (50 mg kg^{-1}) and xylazine (5 mg kg^{-1}) I.M., and then given an initial dose of pentobarbitone (8 mg kg^{-1} I.V.). Anaesthesia was maintained with repeated I.V. bolus injections of pentobarbitone (0·5 mg kg^{-1}) every 30–60 min. A tracheostomy was performed low in the neck in each rabbit and an uncuffed endotracheal tube was tied in place. The rabbits were artificially ventilated at a rate of 20–22 breaths min^{-1} and a tidal volume of approximately 12 ml kg^{-1}. The rabbits were prepared with pneumothoraces, and the expiratory line of the ventilator was placed under 1–2 cm of water to prevent collapse of the lungs. The inspired air was supplemented with O_2 and the arterial P_{O_2} was maintained above 100 mmHg. The arterial P_{CO_2} and pH were maintained in the physiological range by adjusting the tidal volume and by infusing sodium bicarbonate (7·5% (w/v)). The rabbits were paralysed with gallamine (1 mg kg^{-1}) I.V. as needed. The rabbits were allowed to recover from gallamine about every hour, at which time the adequacy of anaesthesia was assessed by testing for the absence of a withdrawal reflex, of an increase in systemic arterial blood pressure, or of an increase in heart rate that occurred either spontaneously or in response to paw pinch. Catheters were introduced into the femoral or jugular vein for infusing anaesthetic and drugs and into the femoral artery for measuring aortic blood pressure and withdrawing blood samples to measure arterial blood gases. Another catheter was inserted into the endotracheal tube and used for monitoring transpulmonary pressure.

To produce pulmonary venous congestion, we opened the pericardium and inserted a cannula made from polyethylene tubing (i.d., 1·67 mm; Intramedic polyethylene tubing, Becton Dickinson, Sparks, MD, USA) with a balloon attached to its distal end into the left atrium through the left atrial appendage. The mitral valve was partially obstructed by inflation of the balloon. A second cannula (i.d., 1·67 mm) was also inserted into the left atrium through the appendage for recording left atrial pressure. Increases in left atrial pressure of 5 mmHg above baseline values were considered to produce mild levels of pulmonary venous congestion. To establish near-threshold levels of pulmonary venous congestion the mean left atrial pressure was increased by 2 mmHg.

For recording RAR activity, the left cervical vagus nerve was placed on a dissecting platform in a pool of mineral oil. Afferent activity was recorded from nerve bundles dissected away from the vagus nerve. The contralateral vagus nerve was left intact. A nerve bundle containing an RAR afferent fibre was split down so that the fibre was the only active fibre discernible or whose signal-to-noise ratio was sufficient to differentiate its activity from the noise by use of a window discriminator. The RARs were identified by their rapid adaptation to a fast rising then maintained hyperinflation (~2–3 tidal volumes) (Widdicombe, 1954). The receptors were localized at the end of each experiment by careful probing of the lungs and airways.

Protocols

Effect of Substance P on baseline RAR activity. Sequential doses of substance P were given every 5 min in one-half log increments until a dose was reached that increased RAR activity by at least 100%. This dose was termed SPIII. Higher doses of substance P were not given because of profound effects on blood pressure. The subthreshold dose was considered the highest dose that produced a <10% increase in RAR activity. Twelve RARs received two sequential doses of substance P; ten of those twelve RARs received three doses.

Effect of NK_1 receptor blockade on RAR responses to substance P and to mild levels of pulmonary venous congestion. In nine RARs that received at least two doses of substance P, the RAR responses to the two highest doses of substance P and to mild levels of pulmonary venous congestion (5 mmHg increases in left atrial pressure maintained for 60 s) were compared before and after I.V. administration of the non-peptide, high-affinity NK_1 receptor antagonist, CP 96345 (Pfizer, Groton, CT, USA). Two additional RARs, in which substance P was not given, were also tested for responses to mild levels of pulmonary venous congestion before and after administration of the NK_1 receptor antagonist. The responses of two other RARs to pulmonary venous congestion were examined before and after injection of 400 nmol kg^{-1} of the inactive (2*R*,3*R*) enantiomer of CP 96345, referred to as CP 96344 (Pfizer). In all animals, the responses of the RARs to substance P and to increases in left atrial pressure were repeated, beginning 20 min after injection of the antagonist or the inactive enantiomer.

From pilot experiments, we determined that 400–600 nmol kg^{-1} CP 96345 was necessary to significantly attenuate the substance P-induced increases in RAR activity when the peptide was given at one-half and one log increments over the threshold dose. We did not exceed this dosage range for the antagonist since higher doses (~10-fold higher) have been associated with effects on L-type calcium channels, unrelated to antagonism of NK_1 receptors (Snider *et al.* 1991; Wang & Håkanson, 1992).

Effect of subthreshold doses of substance P on RAR activity during near-threshold levels of pulmonary venous congestion. For seven RARs (which were among those tested with at least two doses of substance P and to mild levels of pulmonary venous congestion (5 mmHg increases in left atrial pressure)), we compared the effect of subthreshold doses of substance P (the largest doses that increased RAR activity by <10%) on RAR activity against a background of near-threshold levels of pulmonary venous congestion (2 mmHg increases in left atrial pressure).

Data analysis

Group data are expressed as means ± S.E.M. Significance levels were set at $P < 0.05$. To determine the responses of the RARs to graded doses of substance P, RAR activity was averaged over an initial control period (60 s), following each dose of substance P (30 s), and during a final control period (60 s). RAR activity was expressed in impulses per bin. Each bin was 3 s. Dose–response curves were analysed with ANOVA for repeated measures, followed by Fisher's LSD *post hoc* test when appropriate. To determine the effect of NK_1 receptor blockade on the substance P-evoked increases in RAR activity, the dose–response curves to substance P before and after NK_1 receptor blockade were analysed with ANOVA for repeated measures with two within factors (treatment and dose).

To determine the effect of NK_1 receptor blockade on the RAR responses to 5 mmHg increases in left atrial pressure, RAR activity was averaged over an initial control period (60 s), during the increase in left atrial pressure (60 s), and when the increase in left atrial pressure was relieved (60 s) before and after NK_1 receptor blockade. To determine the effect of the combination of subthreshold doses of substance P and near-threshold (2 mmHg) increases in left atrial pressure, RAR activity was compared during an initial control period (60 s), after substance P alone (15 s), during the increase in left atrial pressure alone (60 s), during the combination (15 s), and during a final control period (60 s). The increases in RAR activity during the experimental interventions were compared before and after NK_1 receptor blockade by using ANOVA for repeated measures, followed by Fisher's LSD *post hoc* test when appropriate. Peak changes in arterial blood pressure and peak tracheal pressure were analysed for the two highest doses of substance P before and after NK_1 receptor blockade.

RESULTS

The data are based on sixteen RARs in which complete or partial protocols were completed. One RAR was recorded per rabbit, and all were localized to the upper or middle lobe of the lung. At the commencement of the recordings, mean arterial blood pressure, mean left atrial pressure, heart rate and peak tracheal pressure were 70 ± 3 mmHg, 2·59 ± 0·22 mmHg, 204 ± 7 beats min^{-1} and 3·28 ± 0·29 mmHg, respectively. The pH, P_{CO_2} and P_{O_2} were 7·42 ± 0·05, 38·4 ± 4·4 mmHg and 392 ± 80 mmHg, respectively.

Effect of substance P on baseline RAR activity

Substance P produced dose-dependent increases in RAR activity. The threshold dose for increasing RAR activity ranged from 0·03 to 0·1 $\mu g\ kg^{-1}$; the maximum dose ranged from 0·3 to 3 $\mu g\ kg^{-1}$. The grouped data from the ten RARs that were tested with three doses are summarized in Fig. 1. The mean RAR responses to each of the two highest doses of substance P were significantly different from each other (6·4 ± 1·7 (SPII) *vs.* 11·0 ± 3·1 impulses bin^{-1} (SPIII)) and from baseline activity (1·3 ± 0·5 impulses bin^{-1} (Control)). The mean RAR response to SPIII was also different from that produced by the lowest dose of substance P tested (SPI; 4·2 ± 1·2 impulses bin^{-1}).

Effect of NK_1 receptor blockade on RAR responses to substance P and to mild levels of pulmonary venous congestion

An example of NK_1 receptor blockade on the increase in RAR activity produced by 0·3 $\mu g\ kg^{-1}$ substance P is shown in Fig. 2. Figure 3 shows that the increase in activity of the same RAR to 5 mmHg increases in left atrial pressure was also attenuated by NK_1 receptor blockade.

The grouped data showing the mean responses of the nine RARs to two doses of substance P before and after NK_1 receptor blockade are shown in Fig. 4*A*. Before NK_1 receptor blockade, substance P increased RAR activity in a dose-dependent manner from 1·3 ± 0·5 impulses bin^{-1} (Control) to 5·9 ± 1·7 (SPII) and to 10·2 ± 2·1 impulses bin^{-1} (SPIII). NK_1 receptor blockade did not significantly change baseline RAR activity, which was 3·2 ± 1·1 impulses bin^{-1} before and 2·3 ± 1·4 impulses bin^{-1} after the NK_1 receptor antagonist was given. However, NK_1 receptor blockade significantly attenuated the substance P-induced increases in RAR activity ($P = 0.003$; treatment by dose). After NK_1 receptor blockade the values were 3·1 ± 1·4 impulses bin^{-1} for SPII and 3·8 ± 1·8 impulses bin^{-1} for SPIII. Injection of 400 nmol kg^{-1} CP 96345 was sufficient to significantly

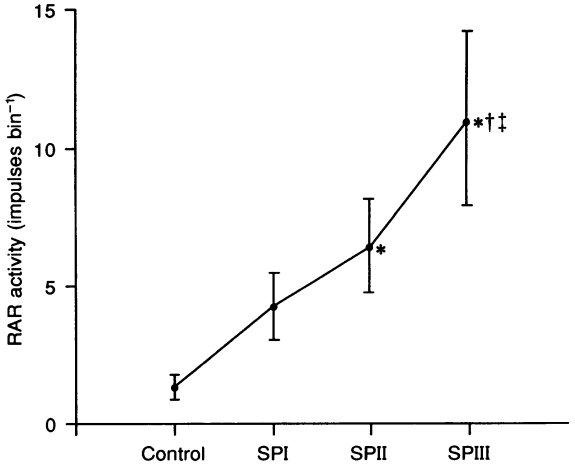

Figure 1. Dose–response curve of RARs to the three highest doses of substance P

Each dose is a one-half log increase above the previous dose; the maximum dose (SPIII) ranged from 0·3 to 3 $\mu g\ kg^{-1}$. RAR responses to the two highest doses (SPII and SPIII) differed from baseline activity (Control) and from each other. RAR responses to SPIII were greater than those produced by SPI. * Significantly different from Control; † significantly different from SPII; ‡ significantly different from SPI. $n = 10$; $P = 0.0008$ by ANOVA for repeated measures; *†‡ $P < 0.05$, Fisher's LSD.

attenuate or block the substance P-induced increases in RAR activity in all but two RARs; in those two, a total of 600 nmol kg^{-1} of the antagonist was required.

The grouped data from eleven RARs showing the mean RAR responses to mild levels of pulmonary venous congestion are shown in Fig. 4*B*. Nine of these eleven RARs were the same as those in Fig. 4*A*. Before NK_1 receptor blockade, 5 mmHg increases in left atrial pressure increased RAR activity from $3{\cdot}8 \pm 1{\cdot}4$ impulses bin^{-1} (Control) to $14{\cdot}7 \pm 3{\cdot}9$ impulses bin^{-1} (LAP + 5). Activity returned to baseline when the increase in left atrial pressure was relieved (LAP off; $4{\cdot}0 \pm 1{\cdot}7$ impulses bin^{-1}). NK_1 receptor blockade did not significantly change baseline RAR activity which was $3{\cdot}8 \pm 1{\cdot}4$ impulses bin^{-1} before and $3{\cdot}5 \pm 1{\cdot}4$ impulses bin^{-1} after NK_1 receptor blockade. However, during NK_1 receptor blockade, the increase in RAR activity following 5 mmHg elevations in left atrial pressure was significantly less than when the manoeuvre was performed before administration of the antagonist (LAP + 5: $14{\cdot}7 \pm 4{\cdot}0$ (pre-) *vs.* $8{\cdot}7 \pm 2{\cdot}2$ impulses bin^{-1} (post-)). Injection of 400 nmol kg^{-1} CP 96345 was sufficient to significantly attenuate or block the increases in RAR activity in all but two RARs; in those two RARs, 600 nmol kg^{-1} of the antagonist was required to attenuate both the substance P- and the pulmonary congestion-induced increases in RAR activity.

The inactive (2*R*,3*R*) enantiomer of the NK_1 receptor antagonist (400 nmol kg^{-1}; CP 96344) had no effect on the increases in RAR activity produced by 5 mmHg increases in left atrial pressure. Pulmonary venous congestion increased RAR activity from $4{\cdot}9 \pm 4{\cdot}8$ to $10{\cdot}8 \pm 9{\cdot}3$ impulses bin^{-1} and from $6{\cdot}0 \pm 6{\cdot}1$ to $13{\cdot}9 \pm 13{\cdot}0$ impulses bin^{-1} before and after administration of the enantiomer, respectively.

At the doses of substance P administered, there were no significant changes in peak tracheal pressure when substance P was administered before or after NK_1 receptor blockade, although there was a trend for peak tracheal pressure to increase over the course of the experiment (Table 1). In most, but not all animals, there was a dose-dependent decrease in arterial blood pressure following application of substance P; the depressor response was significantly less after NK_1 receptor blockade (Table 1). There was no correlation between the magnitude of the change in blood pressure and the change in RAR activity (P = not significant). CP 96345 had no effect on baseline arterial blood pressure or peak tracheal pressure (Table 1).

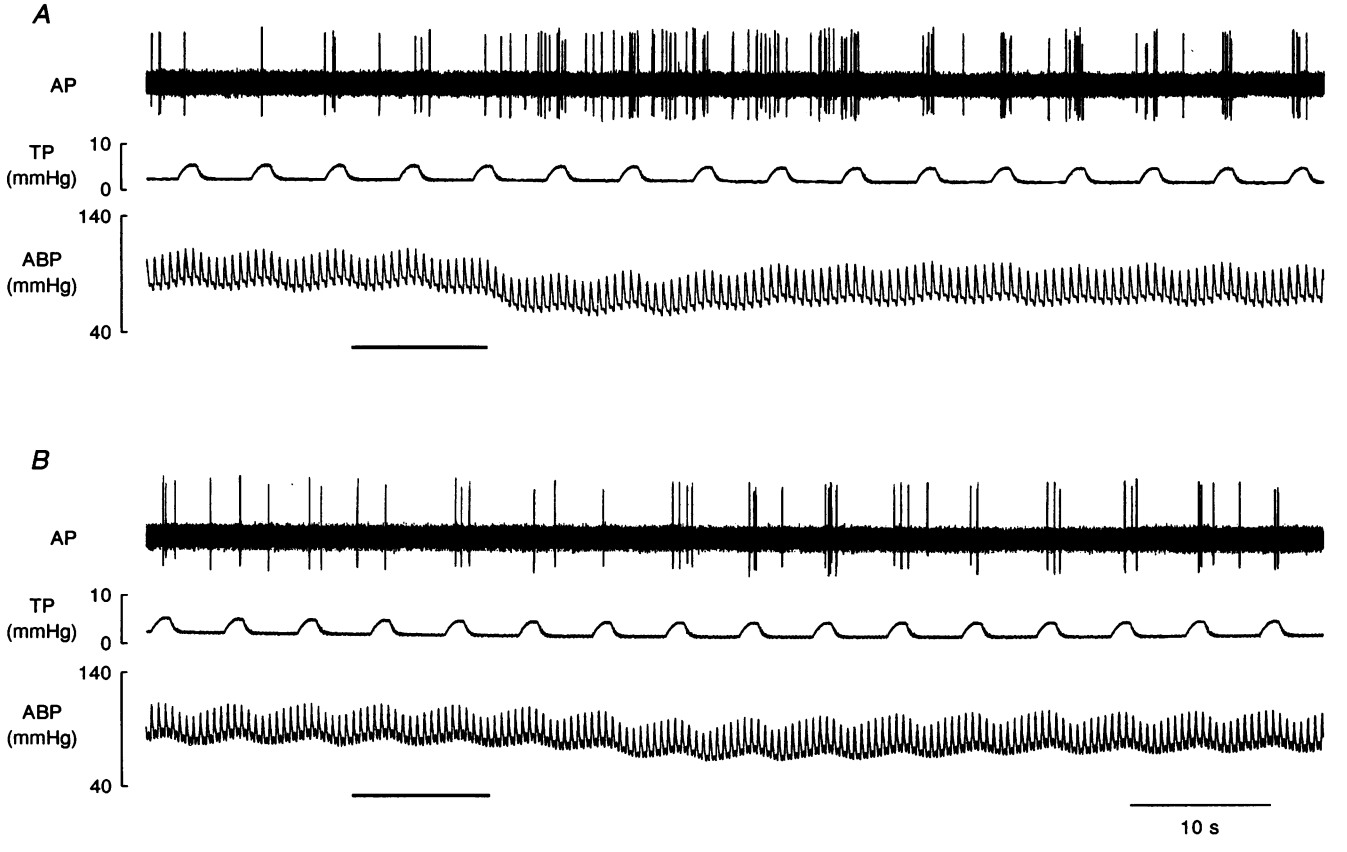

Figure 2. Effect of NK_1 receptor blockade on RAR response to substance P

A, substance P (0·3 μg kg^{-1} I.V.) produced an immediate increase in RAR activity (trace labelled AP) and a small decrease in arterial blood pressure (ABP). *B*, NK_1 receptor blockade with CP 96345 (400 nmol kg^{-1} I.V.) attenuated the RAR response and the decrease in ABP. AP, action potential; TP, tracheal pressure. The horizontal bars indicate the period of injection of substance P.

Table 1. Substance P-evoked changes in arterial blood pressure and peak tracheal pressure before and after NK_1 receptor blockade

	ABP (mmHg)	PTP (mmHg)
SP before NK_1 antagonist		
Control	73 ± 4	3·40 ± 0·46
SPII	59 ± 8*	3·43 ± 0·45
SPIII	54 ± 7*	3·50 ± 0·42
NK_1 antagonist		
Pre-antagonist	70 ± 4	3·78 ± 0·51
Post-antagonist	68 ± 4	3·69 ± 0·59
SP after NK_1 antagonist		
Control	70 ± 4	3·83 ± 0·65
SPII	64 ± 4	3·91 ± 0·70
SPIII	62 ± 4	3·97 ± 0·72

ABP, arterial blood pressure; PTP, peak tracheal pressure; SP, substance P; Control, baseline activity.
* Significantly different from respective Control ($P < 0·05$).

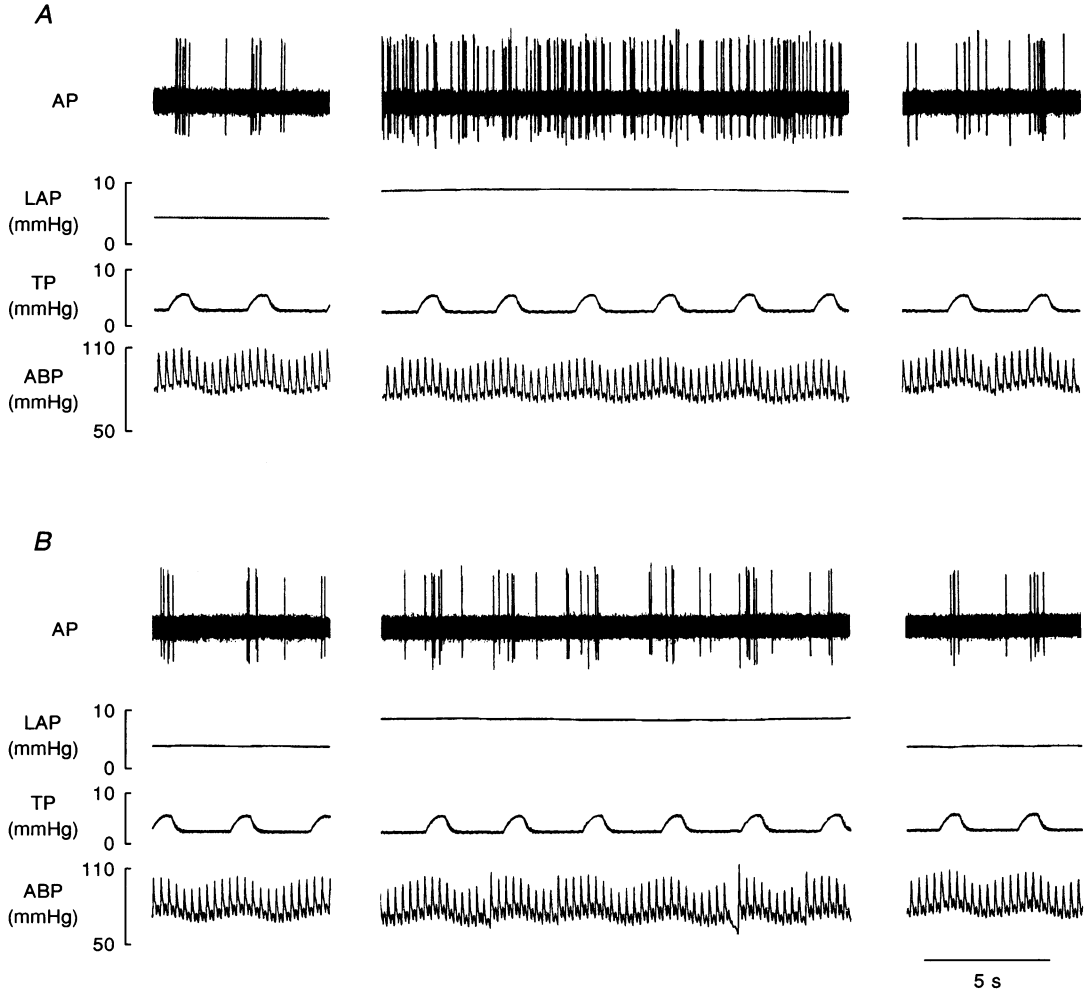

Figure 3. Effect of NK_1 receptor blockade on an RAR response to mild pulmonary venous congestion

A, impulse activity from the same RAR shown in Fig. 2. Mild levels of pulmonary venous congestion (5 mmHg increases in left atrial pressure (LAP)) produced a sustained increase in RAR activity (middle panel). *B*, the increase was significantly attenuated by NK_1 receptor blockade with CP 96345 (400 nmol kg^{-1} I.V.). Abbreviations as in Fig. 2.

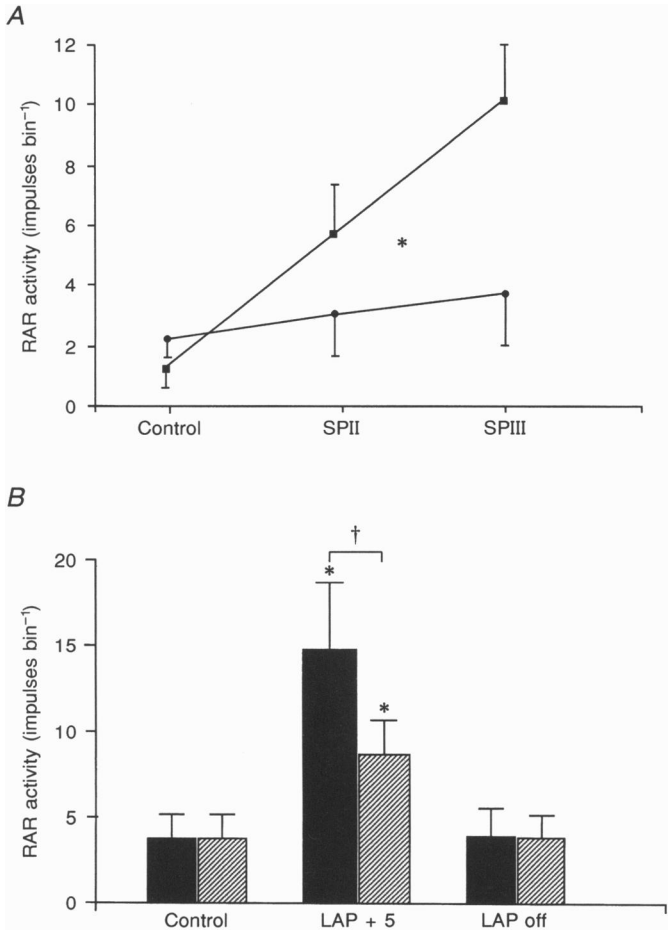

Figure 4. RAR responses to substance P (SPII and SPIII) and to pulmonary venous congestion before and after NK_1 receptor blockade

A, NK_1 receptor antagonism with CP 96345 attenuated substance P-induced increases in RAR activity at both doses ($n = 9$; * $P = 0{\cdot}003$; ANOVA; treatment by dose). ■, pre-NK_1 antagonist; ●, post-NK_1 antagonist. *B*, NK_1 receptor blockade did not alter baseline RAR activity but significantly attenuated the increase produced by pulmonary venous congestion (5 mmHg increases in left atrial pressure; LAP + 5). ■, pre-NK_1 antagonist; ▨, post-NK_1 antagonist. Control, baseline RAR activity; LAP + 5, 5 mmHg increases in left atrial pressure; LAP off, relief of left atrial balloon. * Significantly different from respective Control; † significantly different from each other. $n = 11$; $P = 0{\cdot}0001$ by ANOVA; *† $P < 0{\cdot}05$, Fisher's LSD.

Effect of the combination of near-threshold increases in left atrial pressure and subthreshold amounts of substance P on RAR activity

Figure 5 shows the effect of threshold doses of substance P given at baseline levels and at near-threshold increases (+2 mmHg) in left atrial pressure on RAR activity in seven rabbits. Neither the subthreshold doses of substance P (SPSub) nor the near-threshold levels of pulmonary venous congestion (LAP + 2) had a significant effect on RAR activity. The levels of RAR activity were $3{\cdot}8 \pm 2{\cdot}0$ impulses bin^{-1} during the control period (Control); $3{\cdot}5 \pm 1{\cdot}9$ impulses bin^{-1} after subthreshold doses (SPSub); and $5{\cdot}0 \pm 2{\cdot}2$ impulses bin^{-1} after LAP + 2 mmHg. However, the combination of the two significantly increased RAR activity to $9{\cdot}0 \pm 3{\cdot}3$ impulses bin^{-1} (LAP + 2 + SPSub).

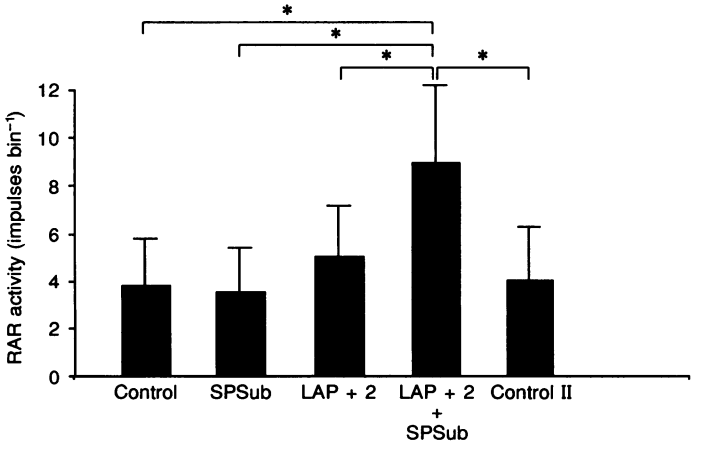

Figure 5. RAR responses to subthreshold doses of substance P (SPSub), near-threshold increases in left atrial pressure (LAP + 2 mmHg) and the combination of the two (LAP + 2 + SPSub)

Neither SPSub nor LAP + 2 changed RAR activity from baseline (Control). LAP + 2 + SPSub significantly increased RAR activity. $n = 7$; $P = 0{\cdot}0001$, by ANOVA; * $P < 0{\cdot}05$, Fisher's LSD. Control II, final control period.

DISCUSSION

The major findings of this study are that (1) substance P stimulates the RARs via an NK_1 receptor-mediated mechanism; (2) NK_1 receptor blockade attenuates the stimulation of RAR activity produced by mild levels of pulmonary venous congestion but does not affect baseline RAR activity at control left atrial pressures; and (3) RAR responses to subthreshold amounts of substance P are enhanced when the peptide is delivered during near-threshold levels of pulmonary venous congestion.

Substance P effects on RAR activity

Substance P produced dose-dependent increases in RAR activity that were significantly attenuated by NK_1 receptor antagonism. At least two previous electrophysiological studies have also shown that substance P stimulates the RARs in rabbits (Prabhakar, Runold, Yamamoto, Langercrantz, Cherniack & Euler, 1987; Matsumoto, Yamasaki, Kanno, Nagayama, Tanno & Shimizu, 1994); however, the present findings specifically implicate NK_1 receptors by the use of a high-affinity selective, non-peptide NK_1 receptor antagonist. Of consideration is the fundamental mechanism(s) by which substance P acting at NK_1 receptors can increase RAR activity. For example: (1) does substance P increase RAR activity by causing microvascular leak; (2) do substance P-evoked increases in mucus secretion stimulate the RARs; (3) does the peptide increase RAR activity by causing bronchoconstriction; or (4) are there NK_1 receptors on the RAR afferent endings which are stimulated directly by substance P?

The following lines of converging evidence seem to support the proposition that substance P stimulates RARs by increasing microvascular leak. Increased microvascular leak is a potent stimulus of RARs (Kappagoda *et al.* 1987; Kappagoda & Ravi, 1989; Ravi & Kappagoda, 1990; Ravi & Kappagoda, 1992; Ravi *et al.* 1994*a*). Exogenous administration of substance P in doses which have been shown to cause airway microvascular leak (Delay-Goyet & Lundberg, 1991; Lembeck *et al.* 1992; Lei *et al.* 1992; Turner, Stow, Hubbs, Gomes & Williams, 1993) increased RAR activity in the current and other studies, an effect that was inhibited by the selective NK_1 receptor antagonist in the current study. Endogenous release of substance P by aerosolized capsaicin has been shown to stimulate the RARs in rabbit, an effect that was diminished by a substance P antagonist (Matsumoto *et al.* 1994). In the guinea-pig, CP 96345, in concentrations similar to those used in the present study, blocked the plasma exudation in the trachea and bronchi produced by vagus nerve stimulation, intravenous capsaicin or substance P, but did not block the bronchoconstrictor effects (believed to be mediated predominantly by NK_2 receptors) produced by vagus nerve stimulation, capsaicin or NKA (Lei *et al.* 1992). Taken together, the data support the argument that substance P increases RAR activity by causing microvascular leak in the airways.

Regarding the other three possible mechanisms for the effects of substance P, it is difficult to demonstrate that substance P-induced mucus secretion, by itself, is a stimulus to the RARs, and to our knowledge there is no such evidence. Nor are there studies that we know of that have demonstrated the existence of NK_1 receptors on RAR terminal endings. It also seems unlikely that substance P-induced bronchoconstriction contributed to the increase in RAR activity, since there were no significant increases in peak tracheal pressure, a global index of airway tone. Even if there were subtle increases in airway tone (which could have been missed by this global index), such increases probably did not contribute substantially to the substance P-evoked increases in RAR activity for the following reason. While the increases in peak tracheal pressure were not statistically significant, the absolute increase in response to the highest dose of substance P (SPIII) before NK_1 receptor blockade was 0·1 mmHg; under those conditions, RAR activity increased by ~700%. After NK_1 receptor blockade, the absolute increase in peak tracheal pressure in response to the same dose of substance P (SPIII) was 0·14 mmHg, and yet RAR activity under those conditions only increased by ~50%.

The two subsequent findings discussed below support the concept that substance P not only stimulates the RARs on its own by increasing microvascular permeability but also amplifies the responses of the RARs to other stimuli that also increase fluid flux.

The contribution of substance P to RAR responses to mild pulmonary venous congestion

The finding that NK_1 receptor antagonism significantly attenuated the increase in RAR activity produced by mild levels of pulmonary venous congestion, suggests that endogenously released substance P contributes to the fluid leak produced mechanically by mild levels of pulmonary venous congestion. The question is what is the source of the substance P during the pulmonary venous congestion? While the RARs are consistently stimulated by mild pulmonary venous congestion (~5 mmHg increases in left atrial pressure), the airway C fibres also modestly increase their activity under these conditions, although to a lesser and more variable extent than the RARs (Kappagoda *et al.* 1987). We propose that this modest stimulation of the airway C fibres causes the local release of sufficient substance P to increase microvascular permeability; the resulting substance P-evoked 'leakiness' then allows an augmented response of the RARs to the hydraulically induced increase in fluid flux caused by pulmonary venous congestion. Thus, substance P released by airway C fibres during pulmonary venous congestion amplifies the mechanically produced microvascular leak. It follows that

NK_1 receptor antagonism should diminish but not abolish the response of the RARs to mild levels of pulmonary venous congestion. Consistent with this is the finding that NK_1 receptor blockade significantly attenuated the increase in RAR activity (by blocking the contribution of substance P), but did not abolish it (the balance of the increase being due to mechanically induced increase in fluid flux).

The proposed model describing the relationships between stimulation of the airway C fibres, substance P release, microvascular leak and RAR stimulation is presented in Fig. 6.

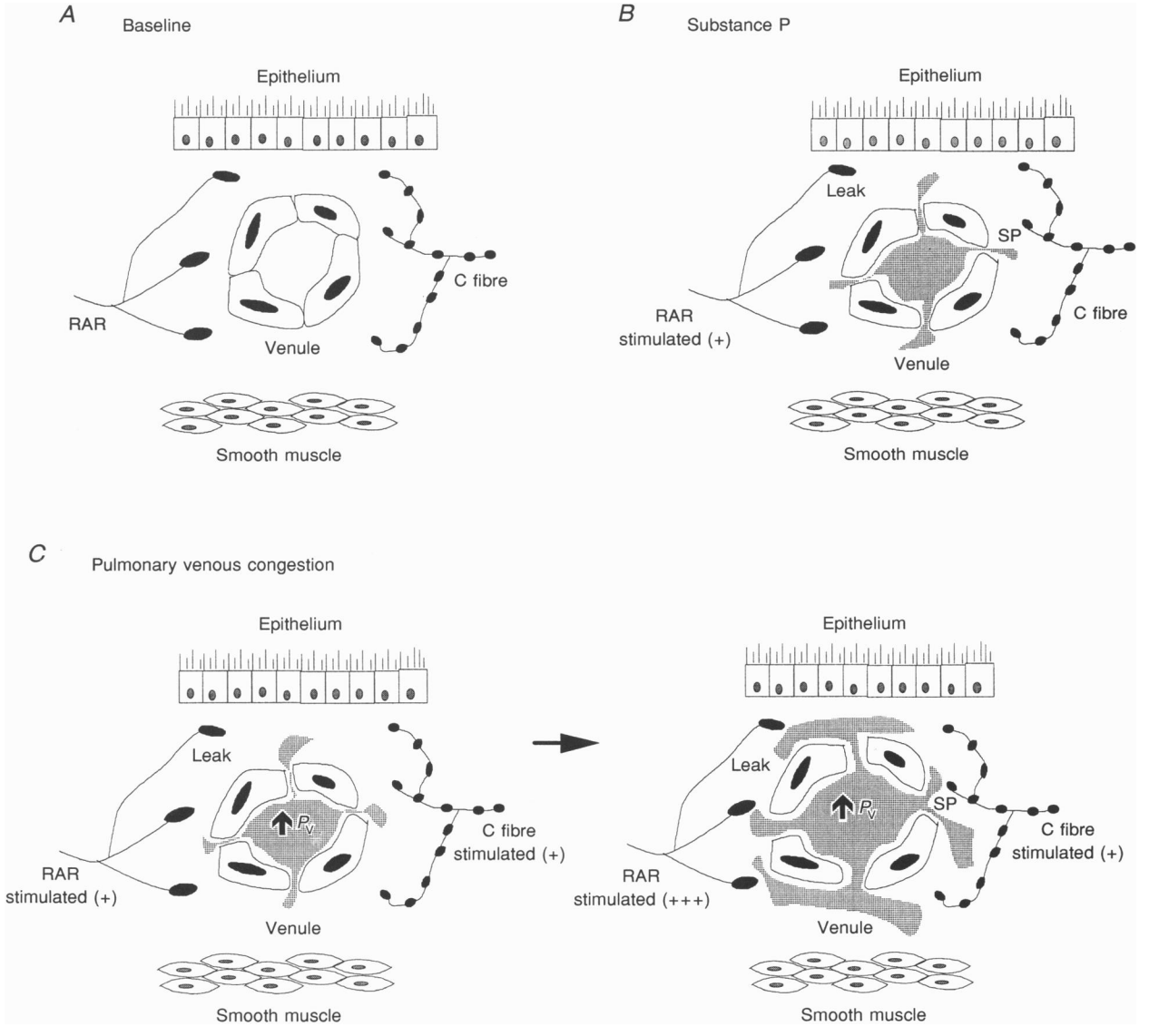

Figure 6. Model for substance P release increase in microvascular leak and the effect on RAR activity

A, afferent C fibres are distributed in both large and small airways within the epithelium and smooth muscle layer and around blood vessels (Maggi *et al.* 1995). RAR endings are also located in the large and small airways near the epithelial layer (Das, Jeffery & Widdicombe, 1978, 1979) and in close apposition to blood vessels (Skepper *et al.* 1990). *B*, stimulation of airway C fibres causes the local release of substance P (SP) which increases endothelial permeability (Nguyen, Villablanca & Rutledge, 1995). The fluid flux into the interstitial space stimulates the RARs. *C*, mild levels of pulmonary venous congestion are associated with modest increases in hydrostatic pressure (P_v) within the lumen of postcapillary venules. The increased P_v causes a mechanically induced microvascular leak that stimulates both C fibres and RARs; the C fibre stimulation results in substance P release; substance P-induced microvascular leak amplifies the hydraulically induced microvascular leak to further stimulate the RARs.

Such an interaction between C fibres and RARs has also been proposed to play a role in the cough reflex. In a recent review article, Widdicombe suggested that the local release of tachykinins by a weak stimulation of C fibres may stimulate the RARs to enhance the cough reflex (Widdicombe, 1995).

Of note also is the recent finding that substance P is contained within encapsulated airway nerve terminals which are presumed to be RARs (Skepper, Kappagoda, McNaughton & Navaratnam, 1990). Although much less is known regarding the functional significance or potential release of substance P from RAR terminals, the localization at least suggests the possibility that an additional source of substance P may be the RARs themselves.

Effect of subthreshold amounts of substance P and near-threshold levels of pulmonary venous congestion

The related finding that doses of substance P, which alone had no effect, stimulated the RARs when delivered during near-threshold levels of pulmonary venous congestion suggests that even meager increases in microvascular permeability (produced by subthreshold amounts of substance P) may set the stage so that, paired with the subtle increases in fluid flux (produced by near-threshold levels of pulmonary venous congestion), the combination stimulates the RARs. This synergistic relationship may extend to other stimuli. For example, cigarette smoke, a potent stimulus of the RARs and airway C fibres, produces increases in microvascular leak that are blocked by CP 96345 or by reduction of C fibres by capsaicin pretreatment (Lundberg, Martling, Saria, Folkers & Rosell, 1983; Delay-Goyet & Lundberg, 1991; Lei, Barnes & Rogers, 1995). Of related interest is the finding that, when cigarette smoke is inhaled against a background of threshold levels of pulmonary venous congestion, it produces an augmented response of the RARs (Ravi *et al.* 1994*b*). Thus, consistent with the model, cigarette smoke stimulates the C fibres to release substance P, which when combined with threshold levels of pulmonary venous congestion provides a more robust stimulus to the RARs. Such an augmented response of the RARs may have functional consequences. We have shown previously that the augmented response of the RAR during near-threshold levels of pulmonary venous congestion resulted in an exaggerated ventilatory response to smoke under the same conditions (Bonham, Ravi; Wilson, Zhang & Kappagoda, 1995).

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