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RSD931, a novel anti-tussive agent acting on airway sensory nerves

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1 The anti-tussive effects, of the local anaesthetic, lidocaine and carcainium chloride (RSD931) have been investigated in guinea-pigs and rabbits.

2 Pre-treatment of guinea-pigs with aerosols of lidocaine or RSD931 at 0.1, 1.0 and 10 mg ml^{-1} reduced the number of citric acid-induced coughs by 9.3, 32.6 and 40.9% ($P>0.05$) for lidocaine and by 25.3% ($P>0.05$), 40.4% ($P>0.05$) and 97.6% ($P<0.01$) for RSD931, respectively and increased the latency to onset of cough at 10.0 mg ml^{-1} only. In addition, RSD931 at 10 mg ml^{-1} reduced citric acid-evoked cough responses in rabbits (with prior exposure to ozone at 3 p.p.m. for 1 h) from 22.1 ± 5.1 to 2.7 ± 0.9 coughs ($P<0.01$).

3 Acute pre-treatment of guinea-pigs with aerosols of lidocaine or RSD931 at 10.0 and 30.0 mg ml^{-1} reduced the number of capsaicin-evoked coughs by 42.2 and 10.3% ($P>0.05$) (lidocaine) and by 25% ($P>0.05$) and 76.9% ($P<0.01$) (RSD931), respectively. Lidocaine had little effect on the latency of cough onset at either 10.0 or 30.0 mg ml^{-1}, however, RSD at 30.0 mg ml^{-1} significantly ($P<0.05$) prolonged the latency of cough onset.

4 RSD931 (10.0 mg ml^{-1}) significantly ($P<0.05 - <0.01$) reduced the spontaneous and histamine-evoked discharges in Aδ-fibres originating from airway, rapidly adapting stretch receptors (RARs) without affecting histamine-evoked bronchoconstriction. Lidocaine at 10.0 mg ml^{-1} also significantly ($P<0.05$) inhibited the spontaneous and histamine-induced discharges of RARs without affecting histamine-evoked bronchoconstriction.

5 Aerosols of RSD931 (10.0 mg ml^{-1}) caused a transient, but significant ($P<0.05$), activation of pulmonary C-fibre endings 2.5 min after administration started. RSD931 had no significant ($P>0.05$) effects on discharges in bronchial C-fibres originating from bronchial C-fibre endings, capsaicin-evoked discharges of either pulmonary or bronchial C-fibre endings or on capsaicin-evoked bronchoconstriction. In contrast, lidocaine (10.0 mg ml^{-1}) significantly ($P<0.05$) inhibited spontaneous and capsaicin-induced discharges in both pulmonary and bronchial C-fibres respectively. Lidocaine also significantly ($P<0.05$) reduced capsaicin-evoked bronchoconstriction.

6 These studies suggest that the anti-tussive actions of RSD931 are mediated *via* inhibition of discharges in Aδ-fibres originating from airway RARs. The mechanism of action of RSD931 is distinct from that of the local anaesthetic lidocaine and RSD931 may represent a novel class of anti-tussive agent.

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Abbreviations: RSD931, carcainium chloride

Introduction

The physiological role of cough is to prevent the aspiration of foreign materials and excess secretions within the respiratory tract, as well as providing a powerful defence mechanism to remove irritant stimuli. However, chronic persistent cough is a common problem in clinical practice and the most common symptom of a wide range of respiratory diseases, including asthma, reflux disease, lung tumours, and following viral and bacterial infections of the respiratory tract (Karlsson & Fuller, 1999). Whilst it is clear that if the underlying disease is identified and appropriately treated, the cough will often disappear, there remains a significant cohort of patients for whom no specific cause of the cough can be found, despite detailed investigations. Indeed it has been reported that chronic non-productive cough having no identifiable cause can account for up to 40% of patients presenting to special cough clinics (Irwin *et al*., 1981). Since persistent cough is often debilitating and embarrassing, for example in business meetings, concert halls or the theatre, there is a clear need for a safe and effective anti-tussive agent.

The opiate codeine, and its derivatives, although frequently used as anti-tussive agents clearly have many other unwanted effects. Other non-opioid drugs having some anti-tussive activity include levodroproprazine, nedecromil sodium, sodium cromoglycate and frusemide, although these agents have been used with limited success in the clinical setting (reviewed by Karlsson & Fuller, 1999; Luporini *et al*., 1998). With intractable cough that is unresponsive to conventional

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therapy, even nebulized local anaesthetics such as lidocaine (Trochtenberg, 1994) have been used as drugs of last resort.

The diversity of stimuli that cause cough do so *via* a common mechanism mediated *via* the vagus nerve and originating from sensory nerve endings in the upper and lower respiratory tract. There are three types of sensory nerve ending found in the mucosa of the larynx and tracheobronchial tree that are involved directly/indirectly in cough, the rapidly adapting stretch receptors (irritant; RARs), the slowly adapting stretch receptors (SARs) and C-fibre receptors (pulmonary and bronchial). There is substantial evidence that RARs are involved in the cough reflex, and stimulation of RARs with thin myelinated Aδ-fibres is the most likely cause of coughing originating from within the tracheobronchial tree (Widdicombe, 1954; 1996). The evidence for a direct role for C-fibres is not so clear (Widdicombe, 1996), with some studies suggesting a causative role, while other studies suggesting an inhibitory role (Collier & Fuller, 1984; Forsberg & Karlsson, 1986; Jackson *et al*., 1989; Tatar *et al*., 1988). However, C-fibres may be involved directly in the cough reflex, since stimulation of C-fibre receptors may release tachykinins, which in turn may activate RARs either directly or indirectly, thus enhancing the cough reflex (Widdicombe, 1995).

Local anaesthetics are the most effective anti-tussive drugs (see Karlsson & Fuller, 1999; Trochtenberg, 1994), but they inhibit all nerve activity in the lung, which precludes their routine use. We were interested in understanding whether the anti-tussive activity of quarternary ammonium compounds such as lidocaine could be dissociated from local anaesthetic activity, and as part of a study of a large number of molecules we investigated the molecule carcainium chloride (RSD931) (Figure 1). In the present study we have compared RSD931 with lidocaine, and have also investigated the possible mechanism of action of RSD931 in comparison with the local anaesthetic lidocaine on impulse discharges in vagal afferent Aδ-fibres and C-fibres originating from RARs, and from pulmonary and bronchial C-fibre sensory endings, respectively, in the tracheobronchial tree.

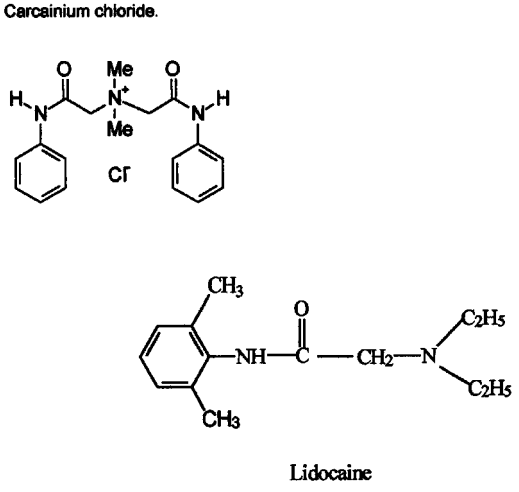

Figure 1 The chemical structures of lidocaine and carcainium chloride.

Methods

The following materials were used during these studies: absolute ethanol (BDH), α-chloralose (Sigma), capsaicin (Sigma), citric acid monohydrate (Sigma), heparin (CP Pharmaceuticals Ltd), histamine diphosphate (Sigma), lidocaine hydrochloride (Sigma), light mineral oil (Sigma), saline (0.85% sodium chloride W/V; Fresenius Ltd), vecuronium bromide (Organon Taknika Ltd), pentobarbitone sodium (Sigma), silver wire (Clark Electromedical Instruments), Tween 80 (Sigma).

RSD931 (carcainium chloride) was synthesized and supplied by Cardiome Pharmaceuticals, Vancouver, Canada.

RSD931, lidocaine and citric acid were dissolved in distilled H_2O. Histamine diphosphate (weight of free base), α-chloralose, pentobarbitone sodium were dissolved in saline. Capsaicin was dissolved in an ethanol, Tween 80 and saline mixture in the ratio 1 : 1 : 23, v v^{-1}. A stock solution of capsaicin (1.0 mg ml^{-1}) was prepared and further dilutions made in saline. All solutions were prepared fresh, each day.

Animals

Male albino Dunkin–Hartley strain guinea-pigs (weight 300–400 g) were supplied by Harlan U.K. Ltd (Bicester, Oxon, U.K.). New Zealand White rabbits (2.4–5.0 kg) of either sex were supplied by Highgate Farm (Nomanby-by-Spital, Market Rasen, Lincolnshire, U.K.) and by Harlan U.K. Ltd.

Anti-tussive activity in guinea-pigs

The method used to assess anti-tussive activity was modified from that described by Adcock *et al*. (1988). Individual conscious guinea-pigs were placed into a sealed purpose-built perspex exposure chamber (3000 cm^3 volume) and allowed to acclimatize prior to the administration of tussive stimuli or drugs by aerosol. Cylinder air (medical grade) was introduced into the exposure chamber at a flow rate of 1 l min^{-1}, maintained by a needle valve and monitored by a ratemeter. From the outset the air passed through the cup of an ultrasonic nebulizer (De Vilbiss Ultraneb 2000) that was used to generate aerosols of either test drug (0.3 ml min^{-1}) or tussive substance (0.5 ml min^{-1}). A Fleisch pneumotachograph (00), connected to a differential pressure transducer (Grass model PTS), was attached to the outflow from the exposure chamber. The differential pressure transducer was connected to a Grass polygraph, from which a hard copy record was produced. The output from the polygraph was input to a computerized data acquisition system (Acqknowledge® – Biopac Systems Inc, Santa Barbara, U.S.A.) for real-time recording of data. A tie-clip microphone was placed in the exposure chamber and connected *via* a pre-amplifier to a loudspeaker output to provide the observer with an audio monitor of cough responses.

Cough responses were induced by exposure to an aerosol of either citric acid (20%, 10 min) or capsaicin (15 μM, 4 min) at flow rates of 2 l and 3 l min^{-1}, respectively. A trained observer continuously monitored animals, and the number of coughs counted over a 15 min period from commencement of the aerosol administration of the tussive stimulus. Three characteristic responses were revealed by

exposure to the two tussive stimuli, cough, sneeze and 'wet dog' shakes. These three types of response were differentiated by sound and visual observation, and confirmation of the number of multiple coughs was determined by reference to the change in flow rate displayed by the AcqKnowledge® system monitor. Data recorded from individual guinea-pigs by the AcqKnowledge® system were stored on 3.5″ diskettes. Each cough was marked on the Grass polygraph paper trace, so that frequencies and the times of onset for coughs could also be determined.

Drugs were weighed and diluted in vehicle, and equal volumes were dispensed into sample tubes before being passed together with another sample tube containing the same volume of vehicle, to an independent observer for blind coding. Pre-treatments were compared with a vehicle control group. Guinea-pigs were randomly allocated to each treatment and animals were pre-treated with vehicle (distilled water), lidocaine or RSD931 for 5 min prior to exposure to aerosols of either citric acid or capsaicin. Lidocaine and RSD931 were administered as aerosols at concentrations of 0.1, 1.0 and 10.0 mg ml^{-1} against citric acid, and 10 and 30 mg ml^{-1} against capsaicin, the sequence of pre-treatment administration being determined according to a Latin square design.

Data are presented as the mean ± s.e.mean of coughs produced by individual guinea-pigs within each group during a 15 min observation period and were analysed using one-way analysis of variance (ANOVA) to compare mean responses between matched groups of animals, followed by the Tukey–Kramer Multiple Comparisons Test. An arbitrary latency end-point of 930 s was assigned to any non-responding animal before statistical analysis of median cough latency by the Kruscal–Wallis non-parametric ANOVA test, whilst a statistical comparison of numbers of responders per group was analysed by Fisher's exact test. Values of $P<0.05$ were considered significant.

Anti-tussive activity in rabbits

New Zealand White rabbits (2.5–3.5 kg) of either sex were placed unrestrained into a sealed purpose-built perspex exposure chamber and allowed to acclimatize prior to exposure of ozone at 3 p.p.m. generated by passing cylinder air through a Sander Ozonizer (Model 25) at a flow rate of 5 l min^{-1} for 1 h. Immediately after exposure to ozone, conscious animals were exposed for 5 min with aerosols (generated with a De Vilbiss Ultraneb 2000 nebulizer) of either vehicle (distilled water) or RSD931 at a nebulization rate of 0.9 ml min^{-1} in a flow rate of 5 l min^{-1}. In a separate series of experiments, immediately after exposure to ozone, animals were treated with intravenously administered saline (0.85% w v^{-1}) or codeine. In both types of experiment, animals were then exposed to an aerosolized solution of citric acid (1.6 M) for 10 min in a flow rate of 5 l min^{-1}. The number of coughs during this period was recorded in the same fashion as that used for guinea-pigs.

Rabbits were randomly allocated to each treatment. Animals were pre-treated with either vehicle (distilled water aerosol; 0.85% sterile saline i.v.), RSD931 aerosol or codeine i.v. for 5 min prior to exposure to aerosols of citric acid. RSD931 was aerosolized as an aerosol concentration of 10.0 mg ml^{-1}, codeine given at 10.0 mg kg^{-1} i.v.

Data are presented as the mean ± s.e.mean of numbers of coughs for individual rabbits in each treatment group during a 10 min observation period. Statistical significance difference was assessed by comparing the group of treated animals to the group of corresponding vehicle controls using the Mann–Whitney *U*-test. Values of $P<0.05$ were considered significant.

Effects of lidocaine and RSD931 on single fibre recordings in anaesthetized rabbits

New Zealand White rabbits of either sex were anaesthetized initially with pentobarbitone, 20 mg kg^{-1} i.v. in a marginal ear vein. Following cannulation of the right jugular vein, anaesthesia was maintained with intravenous α-chloralose at a dose of 60 mg kg^{-1}. If required, anaesthesia was supplemented with up to a further 20 mg kg^{-1} of α-chloralose. The trachea was cannulated with a short length of endotracheal tubing. Airflow was recorded with a heated Fleisch pneumotachograph (size 00) and a Grass differential pressure transducer. Transpulmonary pressure was measured with a second Grass differential pressure transducer *via* an oesophageal balloon positioned in the region of the thorax. The difference between pressure in the oesophageal balloon and that in a side arm of the endotracheal cannula was measured. Blood gases and pH were maintained at physiological levels by artificial ventilation (Palmer ventilator), with a tidal volume of 10 ml kg^{-1} and 48 breaths min^{-1} of laboratory air (Kozma *et al*., 1974). A positive end-expiratory pressure of 2 cm H_2O was used to prevent collapse of the lungs. Animals were paralysed with vecuronium bromide, initially administered at a dose of 0.10 mg kg^{-1} i.v., followed every 20–30 min with 0.05 mg kg^{-1} i.v. to maintain paralysis. Each dose of vecuronium bromide was supplemented with 3 mg kg^{-1} i.v. α-chloralose. The depth of anaesthesia was frequently assessed by monitoring the response of heart rate and blood pressure to noxious stimuli.

An on-line digital pulmonary mechanics analyser (Po-Ne-Mah Gould Instrument Systems Inc, Ohio, U.S.A.), using electronic signals from the differential pressure transducers, calculated the peak values of airflow, tidal volume, transpulmonary pressure, total lung resistance (R_L) and dynamic lung compliance (C_{Dyn}). These variables were recorded continuously, on a breath-by-breath basis.

The right carotid artery was cannulated (Portex 4fg, passed to the ascending aorta/aortic arch) to measure systemic arterial blood pressure using a Statham p23 pressure transducer. Heart rate was obtained from the arterial pulse with a cardiotachometer. Systemic arterial blood pressure and heart rate were continuously recorded on the Po-Ne-Mah data acquisition system and also monitored on a Grass 78B recorder. Body temperature was continuously monitored with a rectal thermometer and maintained at 37–39°C with a heated blanket and control unit.

A cervical vagus nerve (usually the left) was located, *via* a cervical incision, and dissected free from the carotid artery, sympathetic and aortic nerves. The vagus was cleared of its surrounding fascia and cut at the central end. The skin and muscle in the neck at either side of the incision were lifted and tied to a metal ring to form a well, which was filled with light mineral oil. Bipolar plastic coated silver electrodes (exposed at the tips) were used for recording purposes, using fascia positioned on one electrode for a reference. The vagus

nerve was placed on a small black perspex plate to aid subsequent dissection. Thin filaments of nerve were teased from the vagus nerve, under a binocular microscope (Nikon SMZ 1-B), and placed on the second electrode until a single active unit, or one of not more than two or three units, was obtained. Action potentials were recorded in a conventional manner using electrodes connected to a pre-amp headstage (Digitimer NL100K). The signal was amplified (Digitimer NL104), and filtered (Digitimer NL125) before being inputted into a spike processor (Digitimer 130).

The spike processor allowed pulse train counting, with a digital display, over selected time periods. In addition, a digital to analogue converter circuit enabled chart recorder displays of the 'firing rate' of a nerve fibre. Upper and lower discriminator outputs plus a 'window' detector facility allowed, when more than one active unit was present, selection of an action potential if the spike height was sufficiently different from others. Monitoring of the input signal to the spike processor was carried out on a digital storage oscilloscope (Farnell digital storage oscilloscope (DTS 12P), Whetherby, U.K.) and a computerized data acquisition system (EGAA, R.C. Electronics). The input signal to and from the spike processor was also fed through an audio amplifier to a loud speaker. Finally, all the data were recorded on an EGAA data acquisition system and stored for subsequent analysis. All animals were killed at the end of the experiments with an overdose of pentobarbitone.

Conduction velocities were measured in a number of studies to distinguish slow conducting non-myelinated C-fibres from fast conducting myelinated A-fibres. Conduction velocity determinations were carried out by stimulating the vagus nerve close to the thorax with bipolar silver electrodes, using a supra threshold voltage at 0.5 ms, 1 Hz (Grass S44 stimulator). The corresponding action potential was recorded in the nerve fibre under observation. The stimulus and the action potential were captured on the oscilloscope screen in a single sweep. The time interval between the stimulus and recorded action potential was calculated. The distance from the cathode stimulating electrode and the recording electrode was measured with vernier dividers.

Aerosols were generated by a DeVilbiss ultrasonic nebulizer. The nebulizer assembly was connected to the inlet of the ventilator and arranged so that the inspired air passed through the medication chamber and through the Fleisch pneumotachograph before entering the lungs of anaesthetized animals *via* the tracheal cannula.

Criteria for identification of sensory nerve endings

Single vagal nerve fibres were identified as originating from the three groups of airway sensory nerve endings, i.e., slowly adapting stretch receptors (SARs), irritant receptors (rapidly adapting stretch receptors, RARs) and pulmonary and bronchial C-fibre receptors using several criteria (Mohammed *et al*., 1993). These included pattern of spontaneous discharge, response to hyperinflation and deflation, adaptation indices (AIs), response to irritant chemicals after systemic and aerosol administration, conduction velocities and confirmation of location of the receptor in the respiratory tract. Capsaicin (30 μg kg^{-1}) was injected i.v. (jugular vein) or i.a. (ascending aorta/aortic arch) to differentiate between the pulmonary and bronchial C-fibres, respectively. As a rule, a receptor that responded within a latency of 2 s to i.v. capsaicin was considered a pulmonary C-fibre receptor and one that responded within a 2 s latency to i.a. capsaicin was considered a bronchial C-fibre receptor.

After surgery the animals were allowed to stabilize for at least 30 min. After identification of a lung afferent fibre (originating from RARs or C-fibre endings) each animal, and therefore, each nerve, was subjected to the study protocols listed below. Test compounds were administered locally by aerosol *via* the endotracheal cannula at a single dose using a Latin Square design, and their electrophysiological actions evaluated.

Aδ-fibres

In all experiments the spontaneous impulse activity in Aδ-fibres from intrathoracic RARs was recorded for a period of 20 min. Histamine aerosol (six breaths of an aerosol of 1 mg ml^{-1} solution in air) was then administered for two reasons: firstly, as part of the search and identification criteria and secondly, to obtain a standard response of the RAR and lung mechanics to a known RAR activator/bronchoconstrictor agent. The vehicle (distilled H_2O aerosol) was then administered for 5 min and the action potentials were recorded for a period of 20 min following the start of administration. This was followed by an aerosol of the agent under test, either RSD931 (10 mg ml^{-1}) or lidocaine (10 mg ml^{-1}) for 5 min (in separate experiments) and the action potentials were recorded for a period of 20 min following the start of administration. Administration of histamine aerosol, six breaths of a 1 mg ml^{-1} solution was repeated at the end of drug treatment aerosol to assess the effects of either RSD931 or lidocaine on histamine-induced activation of the RAR and histamine-induced bronchoconstriction.

C-fibres

The spontaneous impulse activity in either pulmonary or bronchial C-fibres was recorded for a period of at least 20 min. Capsaicin aerosol (six breaths of an aerosol of 100 μg ml^{-1} solution) was then administered for two reasons: firstly, as part of the search and identification criteria and secondly, to obtain a standard response of the C-fibre ending and lung mechanics to a C-fibre activator/bronchoconstrictor agent. The vehicle (distilled H_2O aerosol) was then administered for 5 min and the action potentials were recorded for a period of 20 min following the start of administration. This was followed by an aerosol of the agent under test, either RSD931 (10 mg ml^{-1}) or lidocaine (10 mg ml^{-1}) for 5 min (in separate experiments) and the action potentials were recorded for a further period of 20 min. Administration of capsaicin aerosol, six breaths of a 100 μg ml^{-1} solution was repeated at the end of the drug treatment aerosol to assess the effects of either RSD931 or lidocaine on capsaicin-evoked activation of the C-fibre ending and capsaicin-evoked bronchoconstriction.

Data analysis

Nerve impulses were counted in 5 s period 'bins' and at each measured time point the average of a 30 s period was

expressed as impulses per s (imp. s^{-1}). Control periods were monitored before administration of each dose of test agent. Impulse discharges (absolute values), before, during and after administration of vehicle (distilled water), RSD931 and lidocaine were averaged (imp. s^{-1}) at -5, 0, 2.5, 5, 10 and 20 min for each individual experiment, and the mean $\pm$ standard error of the mean (s.e.mean) calculated for each group of six experiments. The statistical significance of difference due to test agent treatment was assessed compared to time -5 by two-way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparisons Test. Probabilities (p) of <0.05 were considered significant.

Histamine- and capsaicin-induced changes in airway sensory nerve discharge, C_{Dyn} and R_L are shown as changes (Δ) of absolute changes from baseline values. Variables were pooled and the mean $\pm$ s.e.mean calculated for each group of six experiments. Statistical significance was assessed using Student's *t*-test for paired data. In addition the responses to either histamine or capsaicin aerosol, before and after distilled water, lidocaine and RSD931 were analysed using Student's *t*-test for paired data. Values of $P<0.05$ were considered significant.

Results

Effect of lidocaine and RSD931 on citric acid-induced cough in guinea-pigs

Lidocaine pre-treatment had no significant ($P>0.05$) effect on the time course of cough responses when administered as aerosol solutions of 0.1, 1.0 and 10 mg ml^{-1} but did appear to delay onset of the first cough at 10 mg ml^{-1} (Table 1). Lidocaine appeared to reduce the total number of coughs induced by citric acid over the 15 min observation period, although this effect was not significant (Figure 2, Table 2). The percentage reduction compared with matched vehicle-treated guinea-pigs is also shown in Table 2.

The anti-tussive activity of RSD931 was strikingly different from that for lidocaine. RSD931 prolonged the latency ($P<0.001$) of cough onset at 10 mg ml^{-1} (Table 1). Pre-treatment of guinea-pigs with RSD931 produced a concentration-dependent inhibition of the total number of coughs induced by citric acid over the 15 min observation period (Figure 2, Table 2), which was a highly significant reduction compared with the matched vehicle-treated group ($P<0.001$) and the matched lidocaine group ($P<0.05$) at 10 mg ml^{-1}. The percentage reduction compared with matched vehicle-treated animals is shown in Table 2. At the higher concentration of 10 mg ml^{-1}, RSD931 completely inhibited cough responses in four out of the five guinea-pigs in the group.

Table 1 Mean latency of cough. The effect of aerosol administration of lidocaine and RSD931 on the time (s) to onset of first cough after initiation of citric acid aerosol

Aerosol conc.	*d H_2O*	*Lidocaine*	*RSD931*
0.1 mg ml^{-1}	58 $\pm$ 14	88 $\pm$ 20	88 $\pm$ 6‡
1.0 mg ml^{-1}	94 $\pm$ 19	92 $\pm$ 25	103 $\pm$ 11‡
10 mg ml^{-1}	56 $\pm$ 18[a]	136 $\pm$ 21	778 $\pm$ 137***

Values are mean $\pm$ s.e.mean for $n=5$ or [a]$n=4$ guinea-pigs per group. ‡$P<0.001$, compared to 10 mg ml^{-1}; ***$P<0.001$, compared to distilled H_2O and lidocaine (ANOVA followed by Tukey–Kramer multiple comparisons test). d H_2O = distilled water.

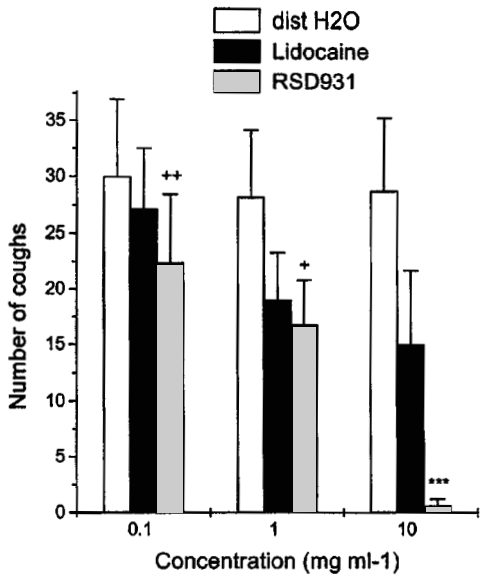

Figure 2 Effect aerosols of distilled H_2O, lidocaine and RSD931 on citric acid-evoked cough responses in conscious guinea-pigs ($n=5$ per group). Results are expressed as total number of coughs in the 15 min period following the start of citric acid aerosol against concentration. Distilled H_2O, lidocaine (0.1–10 mg ml^{-1}) and RSD931 (0.1–10 mg ml^{-1}) were administered by aerosol for 5 min before citric acid. Values are mean $\pm$ s.e.mean in each case. $+P<0.05$ and $++P<0.01$, compared to RSD931 at 10 mg ml^{-1}; ***$P<0.05$, compared to lidocaine and $P<0.001$, compared to distilled H_2O (ANOVA followed by Tukey–Kramer multiple comparison test). dist H_2O = distilled water.

Effect of RSD931 on capsaicin-induced cough in guinea-pigs

Pre-treatment with lidocaine had no effect on the time to onset of coughing at either aerosols of 10 or 30 mg ml^{-1}. Both aerosol concentrations of lidocaine appeared to reduce the total number of coughs induced by capsaicin over the 15 min observation period (Figure 3), but this effect was not statistically significant. RSD931 did not prolong the latency to onset of coughing at 10 mg ml^{-1}, but 30 mg ml^{-1} approximately doubled the mean latency time (317 $\pm$ 84 to 662 $\pm$ 92 s; $P<0.05$). Pre-treatment of guinea-pigs with aerosols of RSD931 reduced the total number of coughs evoked by capsaicin in a concentration-dependent manner (Figure 3). At 30 mg ml^{-1} coughs were reduced from 6.6 $\pm$ 1.2 to 1.5 $\pm$ 0.7 ($P<0.01$).

Effect of RSD931 on citric acid-induced cough in rabbits

Previous unpublished observations in our laboratory have shown that rabbits do not cough consistently to citric acid aerosol. However, when conscious rabbits were exposed to ozone at 3 p.p.m. for 1 h, citric acid aerosol evoked marked

Table 2 Total coughs. The effect of aerosol administration of lidocaine and RSD931 on the total coughs evoked by citric acid in conscious guinea-pigs.

Aerosol conc.	*d H_2O*	*Lidocaine*	*RSD931*
0.1 mg ml^{-1}	30.0±6.9	27.2±5.3 (9.3%)	22.4±6.0‡ (25.3%)
1.0 mg ml^{-1}	28.2±6.0	19.0±4.3 (32.6%)	16.8±4.0† (40.4%)
10 mg ml^{-1}	28.8±6.5[a]	15.0±3.0 (40.9%)	0.6±0.6*** (97.6%)

Values are mean±s.e.mean for $n=5$ or [a]$n=4$ guinea-pigs per group. †$P<0.05$, and ‡$P<0.01$, compared to 10 mg ml^{-1}; ***$P<0.05$, compared to lidocaine and $P<0.001$, compared to distilled H_2O (ANOVA followed by Tukey–Kramer multiple comparisons test). Percentage reduction of the cough response compared with matched vehicle control groups is shown by the values in parentheses. d H_2O = distilled water.

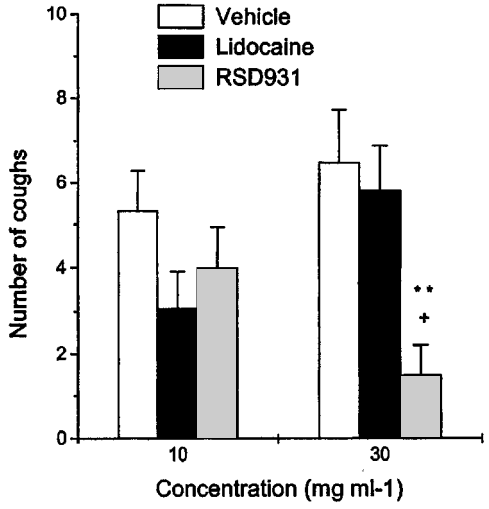

Figure 3 Effect aerosols of distilled H_2O (vehicle), lidocaine and RSD931 on capsaicin-evoked cough responses in conscious guinea-pigs ($n=12$ per group). Results are expressed as total number of coughs in the 15 min period following the start of capsaicin aerosol against concentration. Distilled H_2O, lidocaine (10 and 30 mg ml^{-1}) and RSD931 (10 and 30 mg ml^{-1}) were administered by aerosol for 5 min before capsaicin. Values are mean±s.e.mean in each case. **$P<0.01$, compared to matched vehicle pre-treated group; +$P<0.05$, compared to matched lidocaine pre-treated group (ANOVA followed by Tukey–Kramer multiple comparison test).

cough responses in all rabbits studied (from 0.18±0.18 [1/11] before ozone to 15.9±4.93 coughs [11/11] after ozone exposure) (Gascoigne *et al*., 1999).

In the present series of experiments, in conscious rabbits previously exposed to ozone, citric acid aerosol evoked 22.1±5.1 cough responses in the vehicle treated group. In rabbits pre-treated with aerosols of RSD931 at 10 mg ml^{-1}, cough responses were significantly ($P<0.01$) reduced to 2.7±0.9 (Figure 4). In a separate series of experiments, intravenously administered codeine (10 mg kg^{-1}) significantly ($P<0.05$) inhibited citric acid-evoked cough responses (10.7±2.7 to 3.14±1.3 coughs) when compared to the saline (i.v.) vehicle group.

Effect of lidocaine and RSD931 on single fibre recordings in anaesthetized rabbits

Baseline variables Both RSD931 and lidocaine aerosols were without effect on baseline arterial blood pressure and heart rate throughout the course of the experiments. Likewise, the effects of both agents on baseline pulmonary mechanics were negligible. The values for C_{dyn} were 6.97±0.72 ml cm H_2O^{-1} prior to and 7.03±0.76 ml cm H_2O^{-1} following RSD931, $n=18$; 6.78±0.77 ml cm H_2O^{-1} prior to and 6.89±0.87 ml cm H_2O^{-1} following lidocaine, $n=18$. The values for R_L were 0.041±0.006 cm H_2O^{-1} ml^{-1} s prior to and 0.04±0.007 cm H_2O ml^{-1} s following RSD931, $n=18$; 0.042±0.005 cm H_2O ml^{-1} s prior to and 0.039±0.006 cm H_2O ml^{-1} s following lidocaine, $n=18$. In all cases $P>0.05$.

Aδ-fibres Twelve intrathoracic RARs were examined in twelve rabbits and divided into two groups of six. The spontaneous discharge rate in the fibres of the group ($n=6$) used to study RSD931 was 2.09±0.32 imp. s^{-1} (range 1.28–3.36 imp. s^{-1}) and in the fibres of the group ($n=6$) used to study lidocaine was 2.58±0.28 imp. s^{-1} (range 1.72–3.68 imp. s^{-1}). The adaptation index of each receptor was 100%. Administration of histamine aerosol (six breaths of 1.0 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of these RARs (3.63±0.35 Δ imp. s^{-1}, $P<0.01$, in the RSD931 group and 3.88±0.77 Δ imp. s^{-1}, $P<0.05$, in the lidocaine group) and caused bronchoconstriction as shown by a fall in C_{dyn} (2.42±0.22 Δ ml cmH_2O^{-1}, from 6.9±0.62 to 4.48±0.76 ml cmH_2O^{-1} absolute values, $P<0.05$ in the RSD931 group and 2.73±0.37 Δ ml cmH_2O^{-1}, from 6.5±0.67 to 3.77±0.49 ml cmH_2O^{-1} absolute values, $P<0.01$ in the lidocaine group) and an increase in R_L (0.037±0.007 Δ cmH_2O ml^{-1} s from 0.025±0.006 to 0.062±0.013 cmH_2O ml^{-1} s absolute values, $P<0.05$ in the RSD931 group and 0.042±0.01 Δ cmH_2O ml^{-1} s, from 0.046±0.012 to 0.088±0.014 cmH_2O ml^{-1} s absolute values,

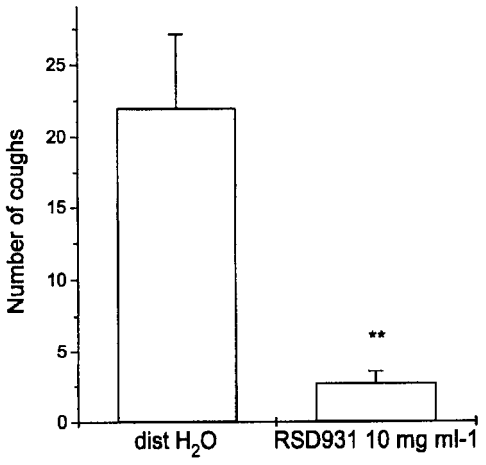

Figure 4 Effect of aerosols of distilled H_2O and RSD931 on citric acid-evoked cough responses in conscious rabbits ($n=11$ per group). Results are expressed as total number of coughs in the 10 min period following the start of citric acid aerosol. Distilled H_2O and RSD931 (10 mg ml^{-1}) were administered by aerosol for 5 min before citric acid. Values are mean±s.e.mean in each case. **$P<0.01$, compared to distilled H_2O (Mann–Whitney U-test).

$P<0.05$ in the lidocaine group). Administration of the vehicle, distilled H_2O aerosols, had no significant effects on the spontaneous discharge of RARs in the RSD931 group, however in the lidocaine group the RARs were significantly ($P<0.05$) activated at 2.5 and 5.0 min after the start of distilled H_2O aerosol (Figure 5). Administration of an aerosol of RSD931 (10 mg ml^{-1} solution) significantly ($P<0.01$) inhibited the spontaneous discharges of RARs from 2.76 ± 0.52 to 0.7 ± 0.1 imp. s^{-1} and 0.41 ± 0.07 imp. s^{-1} at 10 and 20 min after the start of administration, respectively (Figure 5). Similarly, aerosols of lidocaine (10 mg ml^{-1} solution) also significantly ($P<0.05$) reduced the spontaneous discharges of RARs from 2.68 ± 0.27 to 1.35 ± 0.2 imp. s^{-1} and 1.33 ± 0.22 imp. s^{-1} at 10 and 20 min after the start of administration, respectively (Figure 5).

Twenty minutes following the start of administration, both RSD931 and lidocaine significantly reduced the histamine-evoked discharges of the RARs (from 3.63 ± 0.35 to 0.65 ± 0.16 Δ imp. s^{-1}, $P<0.01$, in the RSD931 group and from 3.88 ± 0.77 to 1.78 ± 0.74 Δ imp. s^{-1}, $P<0.05$, in the lidocaine group). However, neither RSD931 nor lidocaine had any significant ($P>0.05$) effects on the histamine-evoked bronchoconstriction (results not shown).

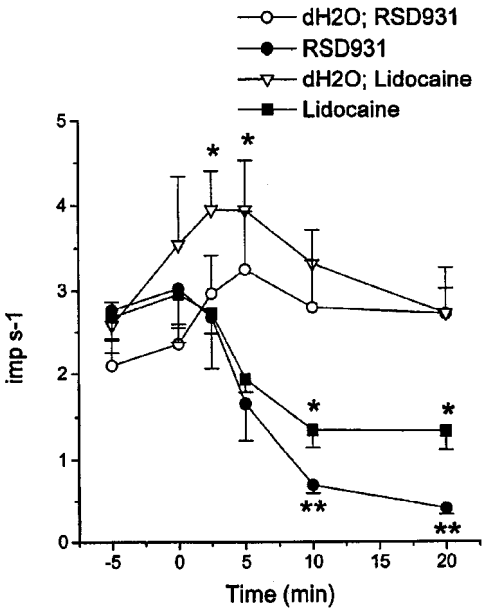

Figure 5 Effect of aerosols of distilled H_2O, lidocaine and RSD931 on discharges in Aδ-fibres originating from RARs in the respiratory tract of anaesthetized, paralysed, artificially ventilated rabbits ($n=12$). Results are expressed as imp. s^{-1} against time (min). Distilled H_2O and lidocaine (10 mg ml^{-1}) were administered sequentially in the one group of animals ($n=6$) at time 0. Distilled H_2O and RSD931 (10 mg ml^{-1}) were administered sequentially in a second group of animals ($n=6$) at time 0. Administration of distilled H_2O aerosols had no significant effects on the spontaneous discharge of RARs in the RSD931 group (0). However, in the lidocaine group the RARs were significantly activated at 2.5 and 5.0 min after the start of the H_2O aerosol dH_2O. Administration of an aerosol of RSD931 or lidocaine significantly inhibited the spontaneous discharges of RARs at both 10 and 20 min after the administration. Values are mean$\pm$s.e.mean in each case. Statistical significance was assessed for each treatment compared to time -5 (ANOVA followed by Dunnett's Multiple Comparison Test), $^*P<0.5$, $^{**}P<0.01$. dH_2O = distilled water.

Bronchial C-fibres

Twelve bronchial C-fibres were examined in twelve rabbits and divided into two groups of six. The spontaneous discharge rate in the fibres of the group ($n=6$) used to study RSD931 was 1.64 ± 0.33 imp. s^{-1} (range 0.68–2.64 imp. s^{-1}) and in the fibres of the group ($n=6$) used to study lidocaine was 2.29 ± 0.65 imp. s^{-1} (range 1.0–5.44 imp. s^{-1}). Administration of capsaicin aerosol (six breaths of 0.1 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of these bronchial C-fibre endings (3.46 ± 1.14 Δ imp. s^{-1}, $P<0.05$, in the RSD931 group and 2.14 ± 0.47 Δ imp. s^{-1}, $P<0.05$, in the lidocaine group), and caused bronchoconstriction as shown by a fall in C_{dyn} (2.4 ± 0.47 Δ ml cmH_2O^{-1} from 7.03 ± 0.6 to 4.63 ± 0.82 ml cmH_2O^{-1} absolute values, $P<0.05$ in the RSD931 group and 2.37 ± 0.66 Δ ml cmH_2O^{-1} from 7.45 ± 0.55 to 5.08 ± 0.7 ml cmH_2O^{-1} absolute values, $P<0.05$ in the lidocaine group) and an increase in R_L (0.018 ± 0.005 Δ cmH_2O ml^{-1} s from 0.069 ± 0.005 to 0.087 ± 0.002 cmH_2O ml^{-1} s absolute values, $P<0.01$ in the RSD931 group and 0.015 ± 0.004 Δ cmH_2O ml^{-1} s from 0.045 ± 0.002 to 0.06 ± 0.004 cmH_2O ml^{-1} s absolute values, $P<0.01$ in the lidocaine group). Administration of the vehicle, distilled H_2O, aerosols had no significant ($P>0.05$) effects on the spontaneous discharge of bronchial C-fibre endings in either the RSD931 group or the lidocaine group (Figure 6). Similarly, administration of an aerosol of RSD931 (10 mg ml^{-1} solution) had no significant ($P>0.05$) effects on the spontaneous discharges of bronchial C-fibre endings (Figure 6). However, aerosols of lidocaine (10 mg ml^{-1} solution) significantly ($P<0.05$) reduced the spontaneous discharges of bronchial C-fibre endings from 2.33 ± 0.36 to 0.99 ± 0.25 imp. s^{-1} and 1.0 ± 0.28 imp. s^{-1} at 10 and 20 min after the start of administration, respectively (Figure 6).

RSD931 had no significant ($P>0.05$) effects on either capsaicin-evoked discharges of the bronchial C-fibre endings or capsaicin-evoked bronchoconstrictor responses (results not shown). Conversely, lidocaine significantly ($P<0.05$) inhibited capsaicin-evoked discharges of the bronchial C-fibre endings from 2.14 ± 0.47 to 0.73 ± 0.23 Δ imp. s^{-1}. In addition, lidocaine significantly ($P<0.05$) reduced capsaicin-evoked bronchoconstrictor responses (C_{dyn} from 2.3 ± 0.66 to 0.68 ± 0.23 Δ ml cmH_2O^{-1}; R_L from 0.015 ± 0.004 to 0.0031 ± 0.001 Δ cmH_2O ml^{-1} s).

Pulmonary C-fibres

Twelve pulmonary C-fibres were examined in twelve rabbits and divided into two groups of six. The spontaneous discharge rate in the fibres of the group ($n=6$) used to study RSD931 was 1.72 ± 0.52 imp. s^{-1} (range 0.26–2.88 imp. s^{-1}) and in the fibres of the group ($n=6$) used to study lidocaine was 2.13 ± 0.43 imp. s^{-1} (range 1.04–3.56 imp. s^{-1}). Administration of capsaicin aerosol (six breaths of 0.1 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of these bronchial C-fibre endings (2.5 ± 0.53 Δ imp. s^{-1}, $P<0.05$, in the RSD931 group and 2.52 ± 0.56 Δ imp. s^{-1}, $P<0.05$, in the lidocaine group) and caused bronchoconstriction as shown by a fall in C_{dyn} (2.25 ± 0.47 Δ ml cmH_2O^{-1} from 7.13 ± 0.56 to 4.88 ± 0.7 ml cmH_2O^{-1} absolute values, $P<0.05$ in the RSD931 group and 2.5 ± 0.87 Δ ml cmH_2O^{-1} from 6.7 ± 0.35 to 4.2 ± 0.76 ml cmH_2O^{-1} absolute values,

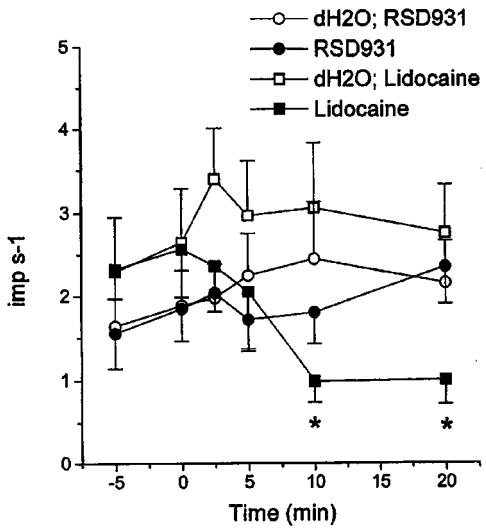

Figure 6 Effect or aerosols of distilled H_2O, lidocaine and RSD931 on discharges in C-fibres originating from bronchial C-fibre endings in the respiratory tract of anaesthetized, paralyzed, artificially ventilated rabbits ($n=12$). Results are expressed as imp. s^{-1} against time (min). Distilled H_2O and lidocaine (10 mg ml^{-1}) were administered sequentially in the one group of animals ($n=6$) at time 0. Distilled H_2O and RSD931 (10 mg ml^{-1}) in a second group of animals ($n=6$) at time 0. Administration of vehicle, distilled H_2O aerosols had no significant ($P>0.05$) effects on the spontaneous discharge of bronchial c-fibres in either the RSD931 or the lidocaine group of animals. Similarly, administration of an aerosol of RSD931 had no significant effects on the discharge of bronchial c-fibres. However, aerosols of lidocaine significantly ($P<0.05$) reduced the spontaneous discharge of bronchial c-fibres at 10 and 20 min after the start of administration. Values are mean$\pm$s.e.mean in each case. Statistical significance was assessed for each treatment compared to time -5 (ANOVA followed by Dunnett's Multiple Comparison Test), $*P<0.5$. dH_2O=distilled water.

$P<0.05$ in the lidocaine group) and an increase in R_L (0.013 ± 0.004 Δ cmH_2O ml^{-1} s from 0.024 ± 0.0041 to 0.037 ± 0.004 cmH_2O ml^{-1} s absolute values $P<0.05$ in the RSD931 group and 0.019 ± 0.005 Δ cmH_2O ml^{-1} s from 0.032 ± 0.006 to 0.051 ± 0.005 cmH_2O ml^{-1} s absolute values, $P<0.05$ in the lidocaine group). Administration of the vehicle, distilled H_2O, aerosols had no significant ($P>0.05$) effects on the spontaneous discharge of pulmonary C-fibre endings in either the RSD931 group or the lidocaine group (Figure 7). However, administration of an aerosol of RSD931 (10 mg ml^{-1} solution) caused a transient but significant ($P<0.05$) activation of pulmonary C-fibres from 1.94 ± 0.21 to 3.44 ± 0.65 imp. s^{-1} at 2.5 min following the start of administration (Figure 7). In contrast, aerosols of lidocaine (10 mg ml^{-1} solution) significantly ($P<0.05$) reduced the spontaneous discharges of pulmonary C-fibre endings from 2.43 ± 0.58 to 1.38 ± 0.027 imp. s^{-1} and 1.21 ± 0.01 imp. s^{-1} at 10 and 20 min after the start of administration, respectively (Figure 7).

RSD931 had no significant ($P>0.05$) effects on either capsaicin-evoked discharges of the pulmonary C-fibre endings or capsaicin-evoked bronchoconstrictor responses (results not shown). In contrast, lidocaine significantly ($P<0.05$) inhibited capsaicin-evoked discharges of the pulmonary C-fibre endings from 2.52 ± 0.56 to 1.29 ± 0.36 Δ imp. s^{-1}. In addition, lidocaine significantly ($P<0.05$) reduced capsaicin-evoked bronchoconstrictor responses (C_{dyn} from 2.5 ± 0.87 to 0.69 ± 0.43 Δ ml cmH_2O^{-1}; R_L from 0.019 ± 0.005 to 0.0057 ± 0.003 Δ cmH_2O ml^{-1} s).

Discussion

The present results demonstrate for the first-time the anti-tussive effects of RSD931 (carcainium chloride), a quaternary ammonium molecule. Nebulized RSD931 inhibited coughing evoked by citric acid in guinea-pigs in a dose-dependent manner. In contrast to nebulized lidocaine, treatment with RSD931 resulted in almost complete inhibition of cough at the highest dose examined. However, many agents have been reported to inhibit citric acid-induced cough in the guinea-pig, and this model is known to identify 'false positives' especially when drugs are administered locally to the airways (reviewed by Karlsson & Fuller, 1999). It is therefore important that RSD931 also caused marked inhibition of coughing induced by citric acid in a second species, i.e., the rabbit, especially since the airways in these animals were rendered hyper-responsive to citric acid following prior exposure to ozone (Gascoigne *et al.*, 1999), an effect equivalent to i.v. codeine in the same model. Furthermore, the anti-tussive action of RSD931 was not confined to inhibition of the coughing evoked by citric acid, since coughing induced by aerosols of capsaicin were also dose-dependently reduced by RSD931 in the guinea-pig.

Coughing is initiated when sensory receptors in the respiratory tract receive stimuli of sufficient intensity to evoke an increase in afferent nerve impulse activity (Widdicombe, 1995; 1996). Cough reflexes can be provoked

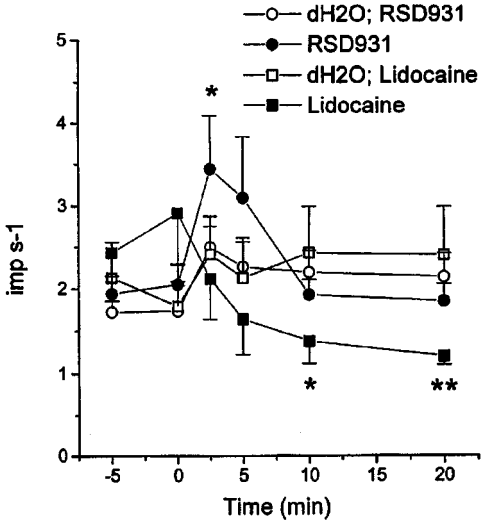

Figure 7 Effect of aerosols of distilled H_2O, lidocaine and RSD931 on discharges in C-fibres originating from pulmonary C-fibre endings in the respiratory tract of anaesthetized, paralysed, artificially ventilated rabbits ($n=12$). Results are expressed as imp. s^{-1} against time (min). Distilled H_2O and lidocaine (10 mg ml^{-1}) were administered sequentially in the one group of animals ($n=6$) at time 0. Distilled H_2O and RSD931 (10 mg ml^{-1}) in a second group of animals ($n=6$) at time 0. Values are mean$\pm$s.e.mean in each case. Statistical significance was assessed for each treatment compared to time -5 (ANOVA followed by Dunnett's Multiple Comparison Test), $*P<0.5$, $**P<0.01$. dH_2O=distilled water.

easily by mechanical and chemical stimuli applied to the epithelium of either the larynx or tracheobronchial tree (Boushey *et al*., 1974; Widdicombe, 1954). Stimulation of RARs (rapidly adapting stretch receptors) in thin myelinated Aδ-fibres is the most likely cause of coughing arising from within the tracheobronchial tree and the evidence for this is widely accepted (Widdicombe, 1996). The contribution of both pulmonary and bronchial C-fibres to coughing is not clear-cut. Some evidence suggests to a causative role, while other evidence suggests an inhibitory role (Collier & Fuller, 1984; Forsberg & Karlsson, 1986; Jackson *et al*., 1989; Tatar *et al*., 1988). Thus inhibition by RSD931 aerosol of both the spontaneous and histamine-evoked discharge in Aδ-fibres originating from rapidly adapting stretch receptors in the tracheobronchial tree of rabbits points to a very obvious peripheral mechanism for the anti-tussive actions of RSD931. The lack of effect of RSD931 on histamine-induced bronchoconstriction in these experiments is not surprising, since histamine contracts airway smooth muscle directly by activating H_1-receptors. The only other classes of compound documented to possess similar activity on Aδ-fibres, which correlated to an anti-tussive action, are the peripherally-acting μ-opioid-receptor agonists (Adcock, 1991; 1999) and the local anaesthetics (Camporesi *et al*., 1979; Cross *et al*., 1976; Karlsson & Fuller, 1999).

The transient stimulation of pulmonary C-fibre receptors by aerosols of RSD931, could possibly contribute to its anti-tussive activity, since stimulation of pulmonary C-fibres by phenylbiguanide and capsaicin in cats inhibits the cough reflex in this species (Tatar *et al*., 1988). Furthermore, the anti-tussive activity of nedocromil sodium in dogs has been attributed to activation of bronchial C-fibres (Jackson *et al*., 1989). The apparent lack of effect of RSD931 on capsaicin-induced discharges of either pulmonary or bronchial C-fibre endings is important since it adds to the increasing evidence that capsaicin does not cause cough by stimulation of C-fibre endings but more likely by activation of irritant receptors (Mohammed *et al*., 1993; Widdicombe, 1996; Canning *et al*., 2000).

In comparison the local anaesthetic lidocaine also reduced the spontaneous and histamine-evoked discharges in Aδ-fibres but differed from RSD931 by also inhibiting the spontaneous and capsaicin-evoked discharges in C-fibres originating from bronchial and pulmonary C-fibre endings. It is possible that this latter effect of lidocaine reduced its effectiveness as an anti-tussive compared to RSD931 in the present experiments by removing an inhibitory gating mechanism involving and maintained by airway sensory C-fibres. Whilst it has been previously reported that RSD931 possesses some local anaesthetic activity, it seems unlikely that the anti-tussive effects of RSD931 observed in our experiments are due to such an action, since RSD931 is much weaker than lidocaine in this respect (Dahlborn & Misiomy, 1965). Thus, in contrast to the local anaesthetic lidocaine, RSD931 inhibited discharges in Aδ-fibres but not in either pulmonary or bronchial C-fibres. Although local anaesthesia in sensory nerves in electrophysiological experiments appears to be an inverse function of fibre diameter, a quite different type of afferent blockade is produced when local anaesthetics are administered by aerosol to the lower respiratory tract. In this case the anaesthetic penetrates from the mucosal surface and blocks the afferent nerve endings located there in an unselective manner. Indeed, in humans, cough evoked with distilled water (Sheppard *et al*., 1983) and by capsaicin (Collier & Fuller, 1984) are both blocked by local anaesthetics. Differential blockade may, however, occur in canine airways, with RARs being blocked more readily than the slowly adapting stretch receptors (SARs, Camporesi *et al*., 1979). Nevertheless, RSD931 selectively blocked RARs (Aδ-fibres) in contrast to the actions of lidocaine, which displayed no selectivity. In addition, lidocaine significantly reduced capsaicin-evoked bronchoconstriction, which was not observed with RSD931. Thus, it seems reasonable to suggest that inhibition of the cough responses by RSD931 observed in the present experiments is unrelated to local anaesthesia but due to either an, as yet, unknown inhibitory mechanism on airway sensory RARs with Aδ-fibres or to an activation of pulmonary C-fibre endings or a combination of the two effects.

Although local anaesthetics are the most consistently effective anti-tussive agnets, which has been demonstrated in a number of studies as inhibition of coughing induced by mechanical irritation and citric acid aerosols in both animals and man (Jain *et al*., 1973; Cross *et al*., 1976; El Khushman *et al*., 1998), the use of nebulized local anaesthetics is not routine except in the treatment of cough during bronchoscopy.

However, local anaesthetics inhibit all neural reflexes, an effect which is clearly not desirable clinically. Furthermore, local anaesthetics such as lidocaine can cause irritation and may precipitate bronchoconstriction, opposing their inhibitory effects on the cough reflex. Since RSD931 is more potent than lidocaine, at least in the guinea-pig, and does not appear to be acting as an anti-tussive by a local anaesthetic action, it may represent a new class of anti-tussive agent with a novel mechanism of action having distinct advantages over local anaesthetics.

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References

ADCOCK, J.J. (1991). Peripheral opioid receptors and the cough reflex. *Respir. Med.,* **85,** 43 – 46.

ADCOCK, J.J. (1999). The relationship between hyperalgesia and hyperresponsiveness in the lung. In *Progress in Inflammation Research: Pain and Neurogenic Inflammation*. eds. Brain, S.D., Moore, P.K. & Parnham, M.J. pp. 195 – 206. Basel, Boston, Berlin: Birkhauser Verlag.

ADCOCK, J.J., SCHNEIDER, C. & SMITH, T.W. (1988). Effects of codeine, morphine and a novel opioid pentapeptide BW443C, on cough, nociception and ventilation in the unanaesthetised guinea-pig. *Br. J. Pharmacol.,* **93,** 93 – 100.

BOUSHEY, H.A., RICHARDSON, P.S., WIDDICOMBE, J.G. & WISE, J.C.M. (1974). The response of laryngeal fibres to mechanical and chemical stimuli. *J. Physiol.,* **240,** 153 – 175.

CAMPORESI, E.M., MORTOLA, J.P. & SANT'AMBROGIO, F. (1979). Topical anaesthesia of tracheal receptors. *J. Appl. Physiol.,* **47,** 1123 – 1126.

CANNING, B.J., REYNOLDS, S.M., MEEKER, S. & UNDEM, B.J. (2000). Electrophysiological identification of tracheal (T) and laryngeal (LX) vagal afferents mediating cough in guinea-pigs (GP). *Am. J. Hosp. Crit. Care Med.,* **161,** A434.

COLLIER, J.G. & FULLER, R.W. (1984). Capsaicin inhalation in man and the effect of sodium cromoglycate. *Br. J. Pharmacol.,* **81,** 113 – 117.

CROSS, B.A., GUZ, A., JAVRI, S.K., ARCHER, S., STEVENS, J. & REYNOLDS, F. (1976). The effect of anaesthetics on the airways in dog and man: a study of respiratory reflexes, sensations and lung mechanisms. *Clin. Sci. Mol. Med.,* **50,** 439 – 454.

DAHLBORN, R. & MISIOMY, A. (1965). Quaternary derivatives of aminoacylanilines. *Acta Pharmacol. Succica,* **32,** 213 – 218.

EL KHUSHMAN, H., MYERS, D., SHAKUR, B.L. & IND, P.W. (1998). Nebulized bupivacaine inhibits capsaicin-induced cough in normal volunteers. *Am. J. Hosp. Crit. Care Med.,* **157** (Suppl): A842.

FORSBERG, K. & KARLSSON, J.-A. (1986). Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea-pigs. *Acta. Physiol. Scand.,* **128,** 319 – 320.

GASCOIGNE, M.H., GARABETTE, M.L., DOUGLAS, G.J., PAGE, C.P. & ADCOCK, J.J. (1999). The effect of pre-exposure to ozone in the cough response to inhaled citric acid in conscious rabbits. *Eur. Resp. J.,* **14** (Suppl.), 157S.

KARLSSON, J.-A. & FULLER, R.W. (1999). Pharmacological regulation of the cough reflex – from experimental models to antitussive effects in man. *Pulm. Pharmacol. Ther.,* **12,** 215 – 228.

KOZMA, C., MACKLIN, W., CUMMING, L.M. & MAUER, R. (1974). Anatomy, physiology and biochemistry of the rabbit. In *The Biology of the Laboratory Rabbit*. eds. Weisbroth, S.H., Flatt, R.E. & Kraus, A.L. pp. 49 – 72. Orlando: Academic Press Inc.

IRWIN, R.S., CORRAO, W.M. & PRATTER, M.R. (1981). Chronic persistent cough in the adult: the spectrum and frequency of causes and successful outcome of specific therapy. *Am. Rev. Respir. Dis.,* **123,** 413 – 417.

JACKSON, D.M., NORRIS, A.A. & EADY, R.P. (1989). Nedocromil sodium and sensory nerves in the dog lung. *Pulm. Pharmacol.,* **2,** 179 – 184.

JAIN, S.K., TRENCHARD, D., REYNOLDS, F., NOBLE, M.I.M. & GUZ, A. (1973). The effect of local anaesthetic administration on the airway respiratory reflexes in the rabbit. *Clin. Science,* **44,** 519 – 538.

LUPORINI, G., BARNI, S., MARCHI, E. & DAFFONCHIO, L. (1998). Efficacy and safety of levodropropizine and dihydrocodeine on nonproductive cough in primary and metastatic lung cancer. *Eur. Resp. J.,* **12,** 97 – 101.

MOHAMMED, S.P., HIGENBOTTAM, T.W. & ADCOCK, J.J. (1993). Effects of aerosol applied capsaicin, histamine and prostaglandin E_2 on airway sensory receptors of anaesthetised cats. *J. Physiol.,* **469,** 51 – 66.

SHEPPARD, D., RIZK, N.W., BOUSHEY, H.A. & BETHEL, R.A. (1983). Mechanism of cough and bronchoconstriction induced by distilled water aerosol. *Am. Rev. Respir. Dis.,* **127,** 691 – 694.

TATAR, M., WEBBER, S.E. & WIDDICOMBE, J.G. (1988). Lung C-fibre receptor activation and defensive reflexes in anaesthetised cats. *J. Physiol.,* **402,** 411 – 420.

TROCHTENBERG, S. (1994). Nebulized lidocaine in the treatment of refractory cough. *Chest,* **105,** 1592 – 1593.

WIDDICOMBE, J.G. (1954). Respiratory reflexes from the trachea and bronchi of the cat. *J. Physiol.,* **123,** 55 – 70.

WIDDICOMBE, J.G. (1995). Neurophysiology of the cough reflex. *Eur. Respir. J.,* **8,** 1193 – 1202.

WIDDICOMBE, J.G. (1996). Sensory mechanisms. *Pulm. Pharmacol.,* **9,** 383 – 387.

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