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A MODEL OF IRRITANT-INDUCED BRONCHOCONSTRICTION IN THE SPONTANEOUSLY BREATHING GUINEA-PIG

C.P. ALLOTT, D.P. EVANS[1] & P.W. MARSHALL

Biology Department, Imperial Chemical Industries Ltd., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG

1 Inhalation of an aqueous aerosol of citric acid caused bronchoconstriction in anaesthetized guinea-pigs which was abolished by bilateral vagal section.

2 Conscious guinea-pigs developed slow, laboured breathing within 90 s of exposure to citric acid aerosol. The onset of this pattern of breathing was delayed by prior aerosol administration of atropine, ipratropium bromide, isoprenaline and tetracaine.

3 The data suggest that exposure of guinea-pigs to citric acid may be a useful model of reflex bronchoconstriction.

Introduction

Bronchoconstriction in asthmatic patients can be provoked by a variety of exogenous and endogenous factors such as pollens, animal danders and stress. The mechanisms by which such a wide variety of stimuli can cause bronchonconstriction are unknown but could include the direct effects of inflammatory mediators released from tissue-bound mast-cells following antigen-IgE combination (Austen & Orange, 1975). However, the work of Altounyan (1964) on the effects of atropine in asthma and the introduction of the atropine analogue, ipratropium bromide, for the treatment of asthma and chronic bronchitis (Poppius, Salorinne & Viljanen, 1972) support the hypothesis that a parasympathetic, bronchoconstricting reflex exists in the lung (Nadel, 1977). Stimulation of this reflex via 'irritant receptors' (Mills & Widdicombe, 1970) has been suggested to be the way in which an asthmatic attack is provoked (Yu, Galant & Gold, 1972).

We have developed a model of irritant-induced bronchoconstriction in guinea-pigs which may be of use in studying effects of repeated challenge on airway calibre and mucus secretion, or the pharmacological control of this reflex. This constriction can be inhibited by the bronchodilator, isoprenaline, the anticholinergic agents atropine and ipratropium bromide, and the local anaesthetic tetracaine.

[1] Present address: Central Toxicology Laboratories, Imperial Chemical Industries Ltd., Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Methods

Lung function in anaesthetized animals

Guinea-pigs (Alderley Park strain) of either sex and weighing 200 to 300 g were used throughout. Each animal was anaesthetized with an intravenous injection of alphaxalone (4.8 mg/kg) and a jugular vein cannulated with polythene tubing (0.05 mm i.d.). A steady level of anaesthesia was maintained by a slow infusion (Scientific and Research Instruments Ltd) of alphaxalone (1.4 mg kg^{-1} min^{-1}). All intravenous injections were given through a 3-way tap in the cannula. A face-mask was placed over the animal's head and air-flow measured by a pneumotachograph and differential pressure transducer (Ether Ltd, UP1). Intra-pleural pressure was measured following insertion into the pleural cavity of a 16-guage needle attached to a saline-filled pressure transducer (Elcomatic, EM780).

Measurements of airway resistance (R_{aw}) and dynamic compliance (C_{dyn}) were made on a breath by breath basis using an analogue computer. Computation was based on the method of Amdur & Mead (1958).

R_{aw} was calculated from the difference in pleural pressure divided by the difference in air flow at the points of half tidal volume. C_{dyn} was calculated as the difference in tidal volume divided by the difference in pleural pressure at points of no-flow. All signals were displayed on a heat-sensitive chart recorder (Devices).

An aerosol of citric acid was generated from a Wright nebulizer powered by compressed air at

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0.21 kg/cm^2 and 20 l/min into a non-pressurized reservoir from which the animal breathed through the face-mask. Changes in resistance and compliance following drug administration were expressed as a percentage rise or fall of the pre-drug levels.

Effects in conscious animals

A guinea-pig was placed in a perspex chamber (15 cm × 15 cm × 30 cm) and exposed to an atmosphere of drug for 2 min. The aerosol was generated from a Wright nebulizer (Aerosol Products Ltd) running at 0.21 kg/cm^2 and 20 l/min of compressed air. The animal was then transferred to an identical chamber either immediately after drug delivery or after an interval as described, and exposed to an aqueous 10% w/v aerosol of citric acid from a glass nebulizer running at 0.28 kg/cm^2 and 32 l/min of compressed air. Both nebulizers delivered particles of 0.5 to 4 μm diameter. The time between the start of the citric acid spray and the end-point of bronchoconstriction was measured in seconds. Bronchoconstriction was characterized by a change from the normal breathing pattern to a slow, laboured breathing with intense abdominal movement. The animal was sprayed with citric acid for 300 s or to the start of bronchoconstriction, whichever was sooner. Drug effects were expressed as the percentage change of time of onset from:

$$((T - C)/(300 - C)) \times 100$$

where T is the time to bronchoconstriction in drug-treated animals and C is the time to bronchoconstriction in placebo-treated animals.

Drugs

The following drugs were used: histamine acid phosphate (BDH Chemicals Ltd, Poole); atropine sulphate (Sigma Chemical Company, Surrey); isoprenaline sulphate (Burroughs Wellcome, Kent); ipratropium bromide (C.H. Boehringer Sohn, Ingelheim am Rhein); tetracaine hydrochloride (Sigma Chemical, Surrey); citric acid, Analar (Hopkin and Williams, Essex); alphaxalone, Saffan anaesthetic injection (Glaxo, Middlesex); dimethyl sulphoxide, Laboratory Reagent (Hopkin and Williams, Essex).

Drugs given by intravenous injection were dissolved in isotonic saline, those given by inhalation were dissolved in dimethyl sulphoxide.

Results

Anaesthetized guinea-pigs

Histamine injected intravenously into anaesthetized guinea-pigs produced a dose-dependent rise in airways resistance and fall in lung compliance. The changes in lung function following 4 μg/kg histamine intravenously (82 ± 5% increase in R_{aw}; 74 ± 6% decrease in C_{dyn}: $n = 10$) were inhibited in a dose-dependent manner by the prior intravenous injection of atropine (10 to 50 μg/kg) or ipratropium bromide (5 to 25 μg/kg).

Citric acid, administered as a 10% w/v aqueous aerosol to guinea-pigs for 1 min, caused an increase in airways resistance of 79 ± 10% and a decrease in

Table 1 Effects of isoprenaline, atropine, ipratropium bromide and tetracaine on citric acid induced bronchoconstriction in conscious guinea-pigs

Treatment	Dose (mg/ml)	Time between sprays (min)	n	Time to bronchoconstriction (s) (mean ± s.e. mean)	Time to bronchoconstriction (% change) (mean ± s.e. mean)	ID_{50} (mg/ml)
Placebo	—	0	16	56 ± 3	—	—
Isoprenaline sulphate	0.005	0	4	139 ± 7	34 ± 3	
	0.010	0	4	203 ± 2	60 ± 1	0.008
	0.025	0	4	268 ± 15	87 ± 6	
Atropine sulphate	0.5	0	4	75 ± 10	8 ± 4	
	1.0	0	4	115 ± 20	24 ± 8	3.19
	5.0	0	4	202 ± 10	60 ± 5	
Ipratropium bromide	1.0	15	4	146 ± 8	37 ± 4	
	5.0	15	4	168 ± 6	45 ± 3	4.18
	10.0	15	4	212 ± 14	64 ± 6	
Tetracaine	5	0	4	114 ± 6	24 ± 3	
	10	0	4	163 ± 16	44 ± 7	11.90
	50	0	3	285 ± 8	94 ± 4	

The drugs were administered by aerosol at the times indicated prior to exposure to citric acid aerosol.

compliance of 68 ± 4% (mean ± s.e. mean, $n = 5$). When administered to animals in which both vagi had been cut, citric acid aerosol caused an increase in resistance of 5 ± 6% and an increase in compliance of 5 ± 9% (mean ± s.e. mean, $n = 5$).

Concious guinea-pigs

Conscious guinea-pigs were exposed to a 10% w/v aqueous aerosol of citric acid and the effects of respiration observed. Within the first 30 s the animals coughed once or twice, and this was followed by a short period of hyperventilation. After about 90 s the animals developed slow, laboured breathing with strong abdominal movements, characteristic of the pre-collapse signs when exposed to carbachol or other bronchoconstricting agents by aerosol. This breathing pattern did not become worse with continued exposure to the irritant, nor did the animals collapse. The time taken from the start of the citric acid spray to the first signs of bronchoconstriction was measured in seconds. To demonstrate that the bronchoconstriction was due to an increase in airways resistance, as seen in anaesthetized animals, groups of guinea-pigs were exposed to an aerosol of the bronchodilator, isoprenaline sulphate, at varying doses immediately prior to citric acid (Table 1). A regression analysis of the $\log_{10}$ dose vs % change in time of onset gave a correlation coefficient $r = 0.997$, and on inverse prediction an ID_{50} (dose giving 50% inhibition) of 0.008 mg/ml. Groups of animals were then exposed to isoprenaline sulphate by aerosol (0.01 mg/ml) and left for various time intervals before exposure to citric acid. Isoprenaline was maximally effective when applied immediately prior to citric acid, its activity decaying to zero by 15 min.

To demonstrate that the bronchoconstriction observed involved parasympathetic innervation, groups of animals were treated with atropine sulphate or ipratropium bromide by aerosol prior to citric acid, and ID_{50} values calculated by regression analysis (Table 1). In contrast to the results following intravenous injection of these agents in anaesthetized animals, ipratropium bromide and atropine were approximately equipotent when given as aerosols.

The local anaesthetic tetracaine, when administered by aerosol immediately before citric acid, also caused a significant dose-dependent inhibition of the bronchoconstriction (Table 1).

The duration of activity of tetracaine (10 mg/ml) and atropine (5 mg/ml) was similar to that of isoprenaline, while ipratropium bromide (10 mg/ml) was most effective when applied 15 min before challenge with citric acid (Figure 1).

All the compounds studied caused a reduction in the number of coughs during exposure to citric acid.

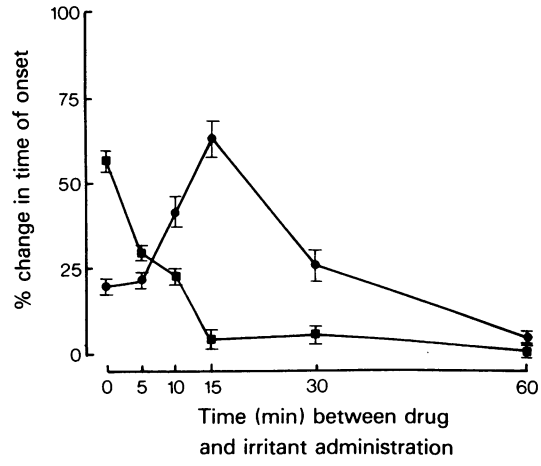

Figure 1 Duration of inhibitory effects of atropine sulphate (■) 5 mg/ml and ipratropium bromide (●) 10 mg/ml expressed as percentage change in time of onset of bronchoconstriction. Animals were exposed to the drugs and then left for the times indicated before exposure to citric acid aerosol. Each point is the mean result of 4 experiments; vertical lines show s.e. mean.

Only tetracaine completely abolished coughing at the doses used.

Discussion

Previous animal models for the study of parasympathetically-induced bronchoconstriction have mainly used histamine as the stimulus. However, controversy exists concerning the extent to which the parasympathetic system is involved in this bronchoconstriction (Gold, Kessler & Yu, 1972; Krell, Chakrin & Wardell, 1976).

The model we describe in this paper circumvents these problems by the use of a non-spasmogenic irritant, citric acid. This had no effect on the guinea-pig isolated trachea (unpublished observations) but caused a marked increase in airways resistance in anaesthetized guinea-pigs. In bilaterally vagotomized anaesthetized animals, citric acid aerosol had no effect on lung function. This demonstrated that bronchoconstriction was mediated by the vagus nerves. In conscious animals, citric acid aerosol caused bronchoconstriction which was inhibited by pretreatment with isoprenaline, showing that the effect was due to airway narrowing. Bronchoconstriction was also inhibited by atropine and ipratropium bromide suggesting that the constriction was parasympathetically mediated. The local anaesthetic, tetracaine, was also effective, presumably by densensitizing pulmonary irritant receptors (Dain, Boushey & Gold, 1975).

Although exposure of guinea-pigs to an aerosol of citric acid is an established model for the study of anti-tussive agents, the usefulness of the model in studying irritant-induced bronchoconstriction has not been described. Coughing was seen in the present experiments and was reduced by pre-treatment with all the drugs used, indicating that both reflexes are intimately related.

Coughing was not observed in the anaesthetized animals presumably due to suppression of the central component of this reflex by the anaesthetic.

The 'hyper-reactivity' or decreased threshold to airway irritation seen in many asthmatic patients may involve stimulation of the parasympathetic nervous system via irritant receptors which can be stimulated by such diverse agents as histamine, carbon dust and antigen (Mills & Widdicombe, 1970; Sellick & Widdicombe, 1971; Dain *et al.*, 1975). Simonsson, Jacobs & Nadel (1967) described the increase in airways resistance in asthmatic patients following inhalation of a 10% w/v aqueous aerosol of citric acid. This effect was presumed to involve a parasympathetic reflex as it was inhibited by pretreatment with atropine. Howard, Cayton, Brennan & Anderson (1977) have treated intractable cough in four patients with aerosol administration of the local anaesthetic lignocaine, possibly acting by desensitizing irritant receptors in the airways. The results we have obtained from guinea-pigs suggest that this model of bronchospasm closely mimics events found in patients with obstructive airways disease.

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