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Muscarinic inhibitory receptors in pulmonary parasympathetic nerves in the guinea-pig

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1 In anaesthetized guinea-pigs, gallamine produced a dose-related potentiation of the bronchoconstriction induced by electrical stimulation of the cervical vagus nerves; (+)-tubocurarine and suxamethonium lacked this effect.

2 The bronchoconstriction produced by intravenous injection of acetylcholine or histamine, however, was not potentiated by gallamine.

3 Vagally-induced bradycardia was abolished by gallamine, confirming antagonism of the effect of acetylcholine on muscarinic receptors in the heart.

4 The muscarinic receptor agonist pilocarpine, had the opposite effect to gallamine in the lung as it inhibited vagally-mediated bronchoconstriction.

5 Pretreatment of guinea-pigs with either guanethidine or propranolol did not affect the gallamine-induced potentiation of vagally-mediated bronchoconstriction.

6 The potentiating effect of gallamine in the lung can be explained by blockade of inhibitory, muscarinic receptors located in the parasympathetic nerves supplying the lungs.

Introduction

The parasympathetic nervous system regulates airway calibre via activation of postsynaptic muscarinic receptors. Recently, the existence of more than one type of muscarinic receptor has been suggested and drugs are now available which show selectivity for different receptor subtypes. Gallamine is known to antagonize the effect of acetylcholine (ACh) on muscarinic receptors in the heart but lacks effects on vascular or gastrointestinal smooth muscle and glands (Riker & Wescoe, 1951; Kennedy & Farham, 1968).

This study was designed to investigate the effects of gallamine on muscarinic receptors in the parasympathetic nervous pathway to the lungs.

Methods

Guinea-pigs (400–600 g) of the Dunkin Hartley strain (Grayston of Hampshire) were used. They were anaesthetized with urethane (Sigma Ltd, 1.50 g kg^{-1}) injected intraperitoneally. The right carotid artery was cannulated for the measurement of blood pressure and a cannula was placed in the left jugular vein for the injection of drugs. Both vagi were cut and the distal end of one nerve was stimulated with shielded platinum electrodes immersed in a pool of liquid paraffin. The animal's body temperature was continually maintained at 37°C using a CFP 8185 homeothermic blanket control.

The animals were paralysed with suxamethonium (succinylcholine chloride, Sigma Ltd, 100 $\mu g\,kg^{-1}$) injected every 8 min and respired with positive pressure, constant volume ventilation from a Harvard Rodent respirator. Airflow ($\dot{V}$) and tidal volume (V_t) were recorded as described by Maclagan & Ney (1979). Tracheal pressure (TP) was measured with a Statham pressure transducer (PM5E): one port of the transducer was connected to a side arm of the tracheal cannula and the other port was open to the atmosphere. All signals were displayed on a Gould Recorder and arterial blood samples were taken at regular intervals and analysed for O_2 and CO_2 tension and pH using a Corning 166 microsample blood gas analyser. Using a tracheal cannula with a resistance of 40–60 $cmH_2Ol^{-1}s^{-1}$, a positive pressure of approximately 80–100 mm of water was needed at each stroke of the pump for adequate ventilation of the animal (Figure 1). Bronchoconstriction was recorded as an increase in TP over the basal insufflation pressure change produced by the pump (Dixon & Brodie, 1903). The sensitivity of the method was

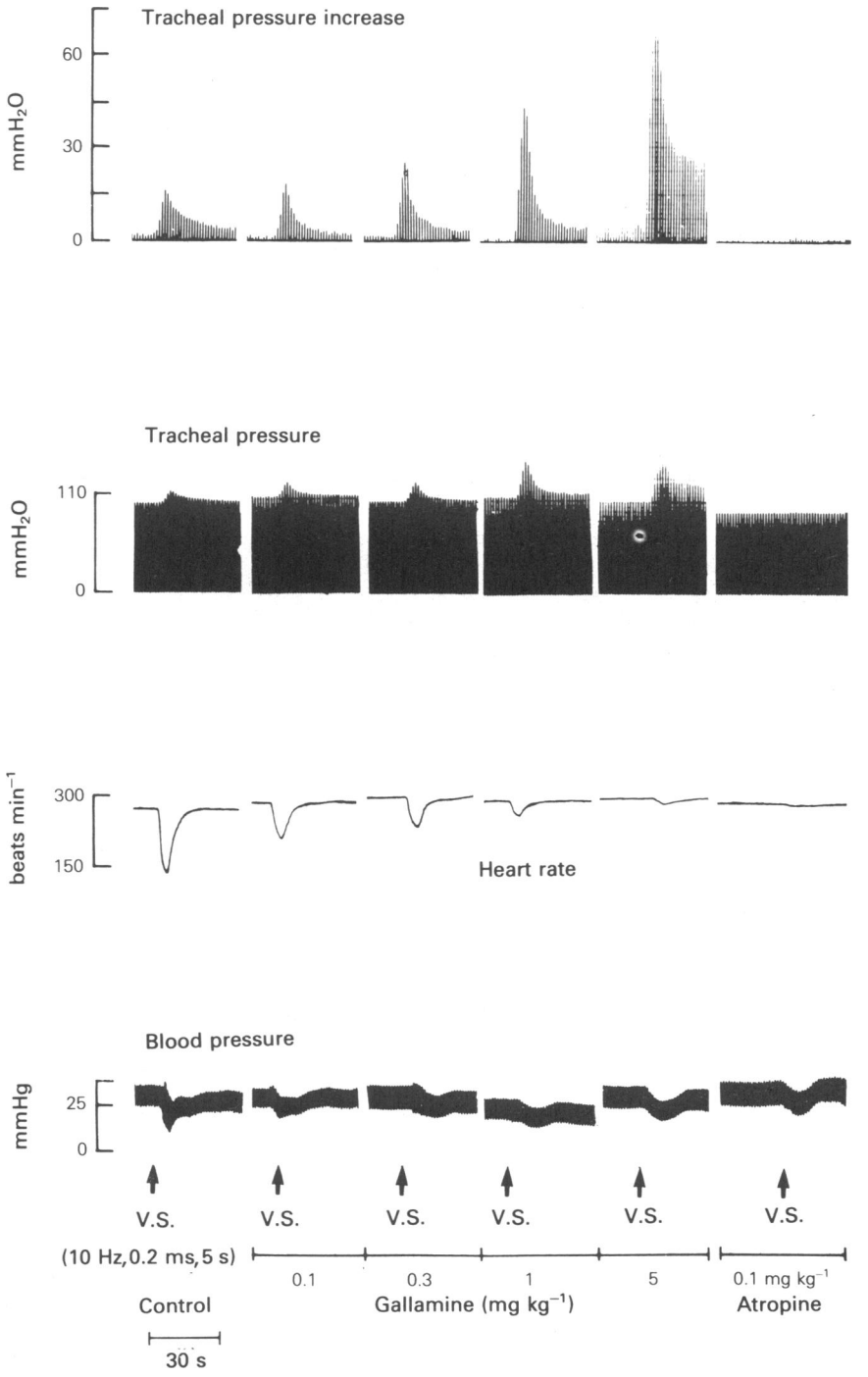

Figure 1 Stimulation of the right vagus nerve (V.S.; 10 Hz, 0.2 ms, 5s) in an anaesthetized guinea-pig paralysed with suxamethonium, caused an increase in tracheal pressure (bronchoconstriction; upper traces) and bradycardia. Gallamine (0.1–5.0 mg kg^{-1}) inhibited the fall in heart rate but potentiated the bronchoconstrictor responses. Atropine (0.1 mg kg^{-1}) abolished the effect of vagal stimulation.

increased by using a computerized device to subtract the basal tracheal insufflation pressure. The subtractor was reset before each nerve stimulus and the increase in TP which occurred during the ensuing bronchoconstriction was recorded on a second channel of the Gould recorder at higher amplification (upper trace, Figure 1). This increased the sensitivity of the method so that reproducible bronchoconstrictor responses could be obtained with stimulation parameters of 2 Hz, 0.2 ms for 20 s which caused an increase in tracheal pressure of between 10 and 20 mmH_2O. The changes in tracheal pressure probably reflect changes in both resistance and compliance components of the lungs.

Drugs

The drugs used in these experiments were; gallamine (May & Baker), +-tubocurarine (Wellcome), guanethidine (CIBA), propranolol hydrochloride (ICI), histamine acid phosphate, pilocarpine nitrate, o-acetylcholine bromide, and atropine sulphate (BDH). All drugs were diluted with saline.

Results

Stimulation of either vagus nerve produced a temporary, frequency-dependent rise in TP (see Figure 1) reflecting changes in both airways resistance and compliance. The right-hand panel of Figure 1 shows that this response was completely abolished by atropine (0.1 mg kg^{-1} i.v.) indicating that it was mediated through muscarinic receptor activation.

Gallamine (0.1 to 10 mg kg^{-1} i.v.) produced a dose-related increase in vagally-mediated bronchoconstriction. Figure 2a shows that the effect was greatest at higher frequencies of stimulation when the response increased ten fold, while the bronchoconstrictor response to stimulation at 2 Hz was barely affected.

Gallamine also produced a dose-related inhibition of vagally-mediated bradycardia which was not frequency-dependent (Figure 2b). This confirmed the antagonist action of gallamine on cardiac muscarinic receptors previously demonstrated in other species.

Figure 3 shows that gallamine did not potentiate the bronchoconstrictor response induced by i.v. injection of ACh (6 μg kg^{-1}) or histamine (4 μg kg^{-1}) indicating that gallamine had not affected the pulmonary postsynaptic muscarinic receptors or altered the muscle tone. In contrast, the response of the heart to both neurally-released and injected ACh was abolished by gallamine.

The opposite effect on the lung was obtained with the muscarinic receptor agonist pilocarpine. Figure 4

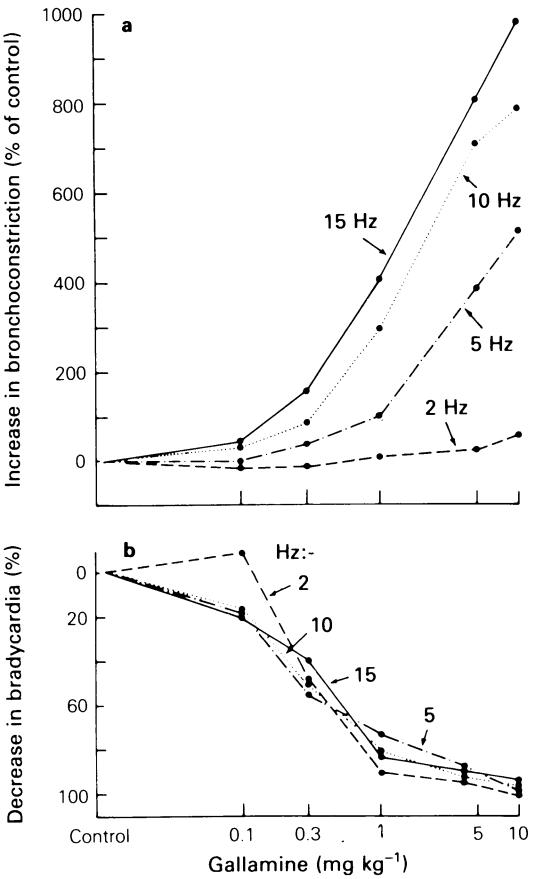

Figure 2 The effect of gallamine (ordinate, dose mg kg^{-1}) on (a) vagally-induced bronchoconstriction and (b) bradycardia. Forty stimuli were delivered in each train at varying frequencies and three measurements were made at each frequency. Results are mean values from 3 animals expressed as percentage change from the response before gallamine.

shows that in doses ranging from 10 μg to 100 μg kg^{-1}, pilocarpine inhibited the bronchoconstriction produced by vagal stimulation and this inhibitory effect was greatest at the lower frequencies of stimulation. Pilocarpine was a weak agonist on the postsynaptic muscarinic receptors on airway smooth muscle; doses of 50 μg kg^{-1} were required to produce a small bronchoconstriction.

The possibility that suxamethonium had depressed the response of the lung to vagal nerve stimulation during the control period was excluded by showing that large doses of suxamethonium (600 μg kg^{-1}) given after gallamine did not alter the response to vagal nerve stimulation.

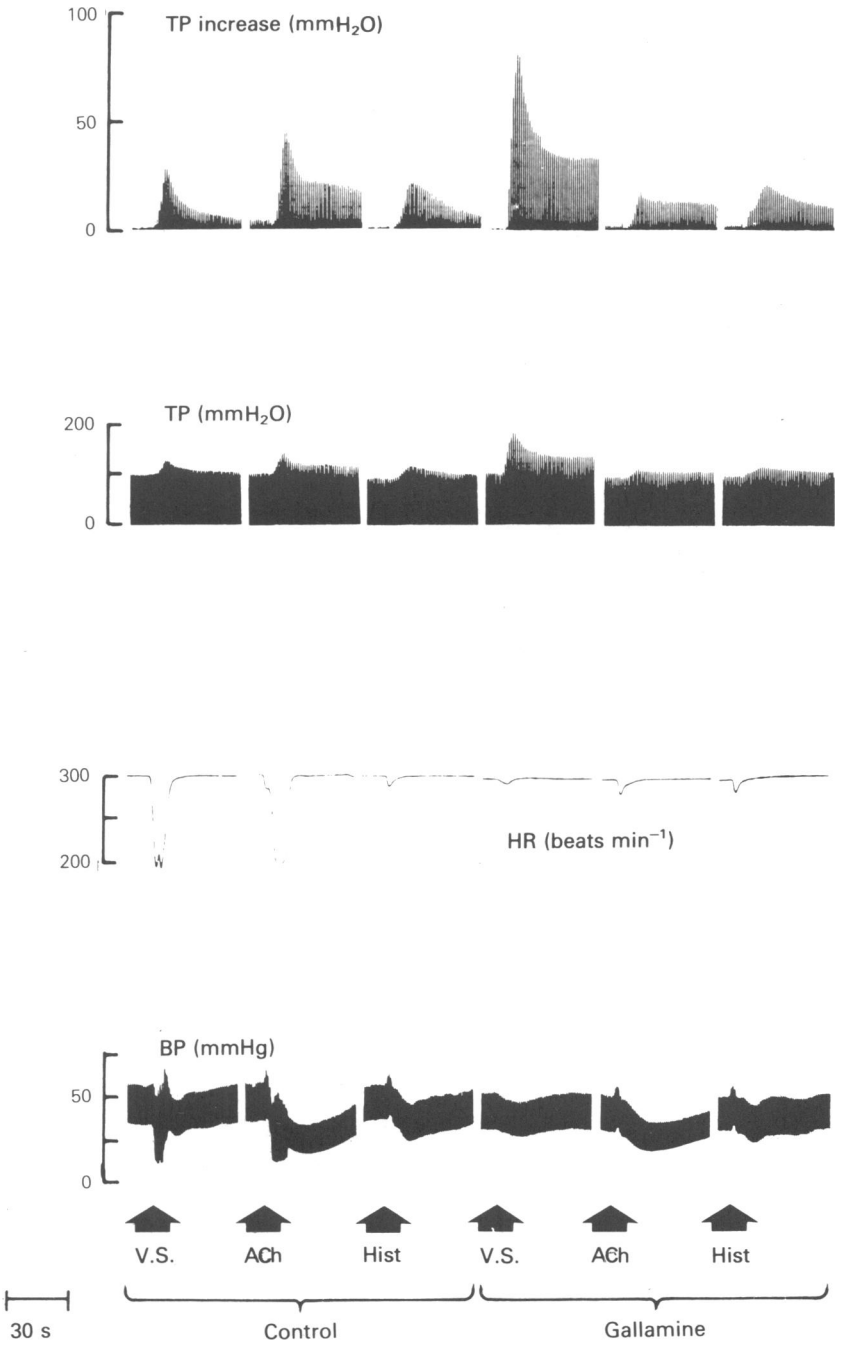

Figure 3 Effects of stimulation of right vagus nerve (V.S.; 10 Hz, 0.2 ms, 4 s), i.v. acetylcholine 5 mg kg^{-1} (ACh), and histamine 4 mg kg^{-1} (Hist) on tracheal pressure (TP), heart rate (HR), and blood pressure (BP) in a guinea-pig anaesthetized with urethane and paralysed with suxamethonium. After gallamine (5 mg kg^{-1}) vagally- and drug-induced bradycardia were abolished, whereas the lung responses to nerve stimulation (TP increase) were potentiated yet responses to injected drugs were not increased.

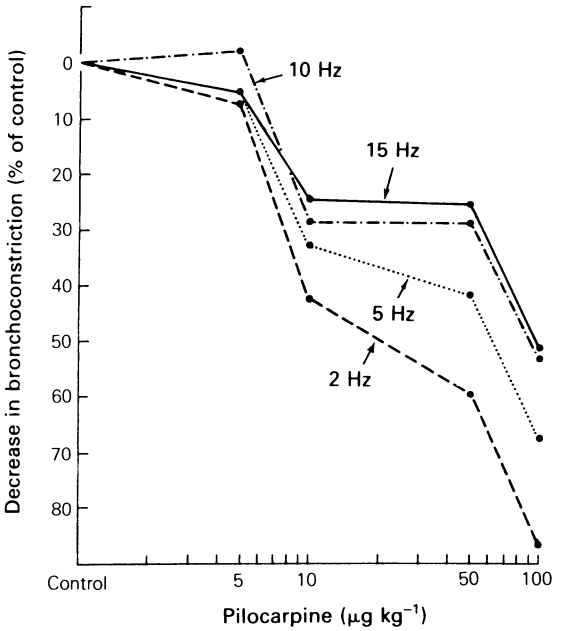

Figure 4 The effect of pilocarpine on the bronchoconstriction induced by vagal nerve stimulation (40 stimuli, 0.2 ms, at 2, 5, 10, and 15 Hz) in anaesthetized guinea-pigs paralysed with suxamethonium. Results are mean values from 3 animals expressed as percentage decrease of the bronchoconstriction before pilocarpine.

The neuromuscular blocking drug +-tubocurarine (1 mg kg^{-1}) also did not affect the bronchoconstrictor or cardiac responses to vagal stimulation, ACh or histamine. Pretreatment of the animals with 4 mg kg^{-1} guanethidine i.v. or 1 mg kg^{-1} propranolol i.v. was also ineffective in preventing gallamine-induced potentiation of vagally-mediated bronchoconstriction.

Discussion

In these experiments, gallamine has been shown to cause potentiation of the bronchoconstriction produced by parasympathetic nerve stimulation without affecting the bronchoconstriction produced by injected ACh. In the heart, however, gallamine abolished the bradycardia induced by both vagal stimulation and injected ACh. Therefore, it is unlikely that this effect of gallamine in the lung is related to cardiovascular changes associated with its ability to abolish vagally-mediated bradycardia. Changes in sympathetic activity due to ganglion blockade can also be excluded because (i) gallamine does not potentiate other bronchoconstrictor agents such as i.v. ACh or histamine, and (ii) tubocurarine, a more potent ganglion blocking drug than gallamine (Bowman & Webb, 1972) does not potentiate vagally-induced bronchoconstriction.

Gallamine is a muscarinic receptor antagonist in the heart (Riker & Wescoe, 1951), in the superior cervical ganglia of the cat (Gardier *et al.*, 1978) and in sympathetic nerves (Leung & Mitchelson, 1983). The results described in this paper may also be due to blockade of muscarinic receptors by gallamine. The finding that the muscarinic receptor agonist, pilocarpine, had the opposite effect to gallamine on vagally-induced bronchoconstriction supports this suggestion.

These receptors cannot be the postsynaptic muscarinic receptors which mediate bronchoconstriction because the effect of injected ACh is not increased by gallamine and the blockade of such receptors cannot explain potentiation of vagally-induced bronchoconstriction. Therefore, the results suggest the presence of muscarinic receptors in autonomic ganglia or peripheral nerve terminals supplying the lung.

Gallamine could produce potentiation of vagally-mediated bronchoconstriction either (1) by blocking excitatory muscarinic receptors in the sympathetic bronchodilator fibres supplying the lungs or, alternatively (2) by blocking inhibitory muscarinic receptors in the parasympathetic nerves to airway smooth muscle.

The first possibility can be excluded because pretreatment with guanethidine or propranolol did not prevent the gallamine-induced potentiation of vagally-induced bronchoconstriction.

The second possibility is more feasible. Muscarinic receptors with an inhibitory function are known to exist in the parasympathetic ganglia of cat bladder (Gallagher *et al.*, 1982) and in parasympathetic nerve terminals in guinea-pig ileum (Kilbinger & Wessler, 1980; Fosbraey & Johnson, 1980) although gallamine has not been tested on these receptors. Our results with gallamine can be explained by blockade of inhibitory muscarinic receptors located in the pulmonary parasympathetic cholinergic nerves. These receptors may have different properties to the 'classical' postsynaptic receptor on airway smooth muscle.

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