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Neuronal muscarinic receptors attenuate vagally-induced contraction of feline bronchial smooth muscle

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1 In anaesthetized cats, stimulation of the vagus nerves produced bradycardia and a bronchoconstriction which was measured as an increase in lung resistance (R_L) and a fall in dynamic lung compliance (C_{dyn}); these effects were abolished by atropine.

2 Gallamine potentiated vagally-mediated changes in R_L and C_{dyn} at doses that blocked muscarinic receptors in the heart and inhibited neuromuscular transmission. (+)-Tubocurarine and suxamethonium did not affect the response of the lung or the heart to vagal stimulation.

3 Bronchoconstriction induced by intravenous acetylcholine was not potentiated by gallamine, indicating that postsynaptic muscarinic receptors in the lung and changes in muscle tone were not involved.

4 Potentiation of vagally-induced bronchoconstriction appears to be due to blockade of inhibitory muscarinic receptors located in the pulmonary parasympathetic nerves innervating both central and peripheral airways.

5 Pilocarpine was an agonist for these neuronal receptors as it inhibited vagally-induced bronchoconstriction at low doses (10 ng to 1 $\mu g\,kg^{-1}$).

6 The results demonstrate that gallamine is an antagonist and pilocarpine an agonist at neuronal muscarinic receptors which attenuate parasympathetic nerve activity in feline lung.

Introduction

Inhibitory muscarinic receptors have been demonstrated in autonomic nerves innervating the intestine (Dzieniszewski & Kilbinger, 1978), blood vessels (Shepherd *et al.*, 1978), heart (Loffelholz & Muscholl, 1970), bladder (Gallagher *et al.*, 1982), and nictitating membrane (Gardier *et al.*, 1974). Similar neuronal receptors have recently been reported to attenuate parasympathetic nerve activity to the guinea-pig lung (Fryer & Maclagan, 1984).

There are important differences in the innervation of cat and guinea-pig airway smooth muscle (Richardson, 1979) and results cannot automatically be transferred from one species to another; for example, neuronal muscarinic receptors do not appear to be present in rat lung (Fryer & Maclagan, unpublished results). It was especially important to know whether muscarinic receptors influence parasympathetic nerve activity in cat lung because this species has been used for many of the definitive studies on the dominant role of parasympathetic reflexes in the control of airway smooth muscle, (Daly & Mount, 1951; Widdicombe, 1954a,b; Nadel *et al.*, 1971; Olsen *et al.*, 1965).

Parasympathetic nerves are known to innervate airway smooth muscle down to the level of the peripheral, respiratory bronchioles (Olsen *et al.*, 1965; Nadel *et al.*, 1971; Cabezas *et al.*, 1971). In order to localize the neuronal muscarinic receptors within the airways, a method was chosen which recorded both lung resistance and dynamic lung compliance as these measurements reflect changes occurring mainly in the central and peripheral airways respectively (Widdicombe, 1963).

Methods

All experiments were performed in cats (2.0 to 4.0 kg), anaesthetized with 70 $mg\,kg^{-1}$ α-chloralose plus 6 $mg\,kg^{-1}$ pentobarbitone sodium intravenously. The right carotid artery was cannulated for the measurement of blood pressure and heart rate (derived from the blood pressure signal). A cannula was placed in the left jugular vein for the administration of drugs. The animals were paralyzed with suxamethonium (10 $\mu g\,kg^{-1}\,min^{-1}$) infused into the right femoral vein

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throughout the experiment or until gallamine was given. All animals were artificially ventilated by means of a Harvard respiration pump.

Both vagi were tied and the distal ends stimulated simultaneously (10–20 V, 0.2 ms, 5–30 Hz, 300 pulses per train) at 5 min intervals with shielded platinium electrodes immersed in a pool of liquid paraffin. Body temperature was maintained at 38.5°C with a homeothermic blanket (CFP 8142). Arterial blood gases were monitored throughout the experiment with a Radiometer Copenhagen BME 33/BGA3 blood gas analyser.

Animals were prepared for the measurement of lung resistance and compliance as described by Maclagan & Ney (1979). The tracheal cannula was connected in series with a Fleish pneumotachograph (size 00) and airflow measured with a differential pressure transducer (Validyne, MP45-14). The flow signal ($\dot{V}$) was integrated to give tidal volume (V_t) and the transpulmonary pressure (P_{tp}) was recorded with a second Validyne which measured the pressure difference between the tracheal cannula and a trocar in the intrapleural cavity.

Lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were derived by the method of Amdur & Mead (1958) with a Buxco Pulmonary Mechanics Analyser Model 6 (Buxco Electronics Inc., Sharon, Conn.) as described by Ney (1983). All signals were recorded on a Gould Brush Polygraph.

Measurement of neuromuscular blockade

Both ends of the tibia were drilled and the limb fixed in the horizontal position to a rigid steel frame. Isometric maximal twitches of the tibialis anterior muscle were elicited by stimulating the peripheral end of the cut sciatic nerve (supramaximal voltage, 0.1 Hz, 0.1 ms) and were recorded by means of a Statham strain gauge, as described by Buller *et al.*, (1960). The initial resting tension of the muscle was adjusted to that value at which the maximal twitch was obtained. The temperature of the muscle was monitored with a temperature probe (Light Laboratories Ltd) and kept constant throughout the experiment at 37°C. In these experiments, control responses had to be carried out at the start of the experiment in the absence of neuromuscular blocking drugs. The cats were artificially respired throughout the experiments.

The drugs used in these experiments were; gallamine triethiodide and pentobarbitone sodium (May & Baker); (+)-tubocurarine chloride (Wellcome); guanethidine sulphate (CIBA); suxamethonium chloride (Sigma); pilocarpine nitrate, 5-hydroxytryptamine creatinine sulphate, acetylcholine bromide, tyramine hydrochloride, atropine sulphate and α-chloralose (BDH). All drugs were diluted with saline.

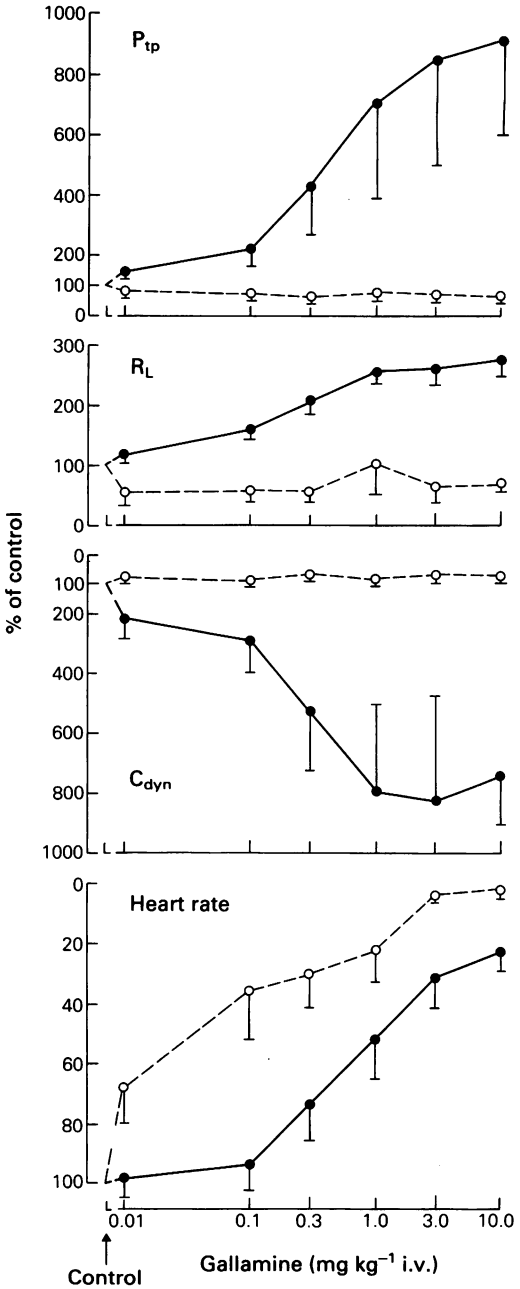

Figure 1 The effect of cumulative doses of gallamine (abscissa scale) on bronchoconstriction and bradycardia elicited by vagal stimulation (●–●) and acetylcholine (O–O) in 4 anaesthetized cats. Absolute values for the control responses (mean ± s.e.mean) to vagal stimulation (10 V, 0.2 ms, 10 Hz, 300 pulses per train) and to 20 μg kg^{-1} acetylcholine (in parentheses) were: increase in transpulmonary pressure, P_{tp}, 0.6 ± 0.2 (1.8 ± 0.3) cmH_2O; increase in lung resistance, R_L, 7.3 ± 1.4 (7.0 ± 1.6) cmH_2O l^{-1} s^{-1}, decrease in dynamic lung compliance, C_{dyn}, 0.82 ± 0.40 (2.79 ± 0.40) ml cmH_2O^{-1}; and fall in heart rate 144.0 ± 10.8 (43.8 ± 11.1) beats min^{-1}. Mean results are given, expressed as a % of the control response obtained before gallamine; vertical lines show s.e.mean.

Results

In 10 cats anaesthetized with α-chloralose and artificially respired, the resting values for lung function were (mean ± s.e.mean): lung resistance (R_L) 7.7 ± 0.3 $cmH_2O\,l^{-1}s^{-1}$, transpulmonary pressure (P_{tp}) 7.6 ± 0.6 cmH_2O, dynamic lung compliance (C_{dyn}) 10.95 ± 0.57 ml cmH_2O^{-1}. The mean heart rate was 186.7 ± 24.3 beats min^{-1}. Stimulation of the vagi produced a fall in heart rate together with bronchoconstriction which was measured as a rise in lung resistance and fall in dynamic lung compliance. The bronchoconstriction produced by vagal nerve stimulation was frequency-dependent and completely abolished by atropine (100 μg kg^{-1}) indicating that it was mediated via cholinergic nerves.

Gallamine (0.1 to 10 mg kg^{-1} i.v.) potentiated the bronchoconstriction induced by vagal nerve stimulation. Figure 1 shows that the increase in the bronchoconstrictor effect occurred both in the central airways (shown by potentiation of the R_L increase) and also in the peripheral airways (shown by potentiation of the fall in C_{dyn}).

The responses of the postsynaptic muscarinic receptors in the airway smooth muscle were tested by eliciting bronchoconstriction with intravenous injections of acetylcholine (ACh, 20 μg kg^{-1}). In Figure 1, the results have been expressed as a % of the bronchoconstrictor responses before gallamine. The changes during the control period in R_L, P_{tp} and C_{dyn} are given in absolute values in the legend to Figure 1. It can be seen that the dose of ACh was chosen to match the change in R_L produced by vagal nerve stimulation. However, as ACh caused a greater effect than vagal stimulation on P_{tp} and C_{dyn} it was impossible to match all three parameters. The marked effect of ACh on compliance and P_{tp} may be due to its vasodilator and hypotensive effects.

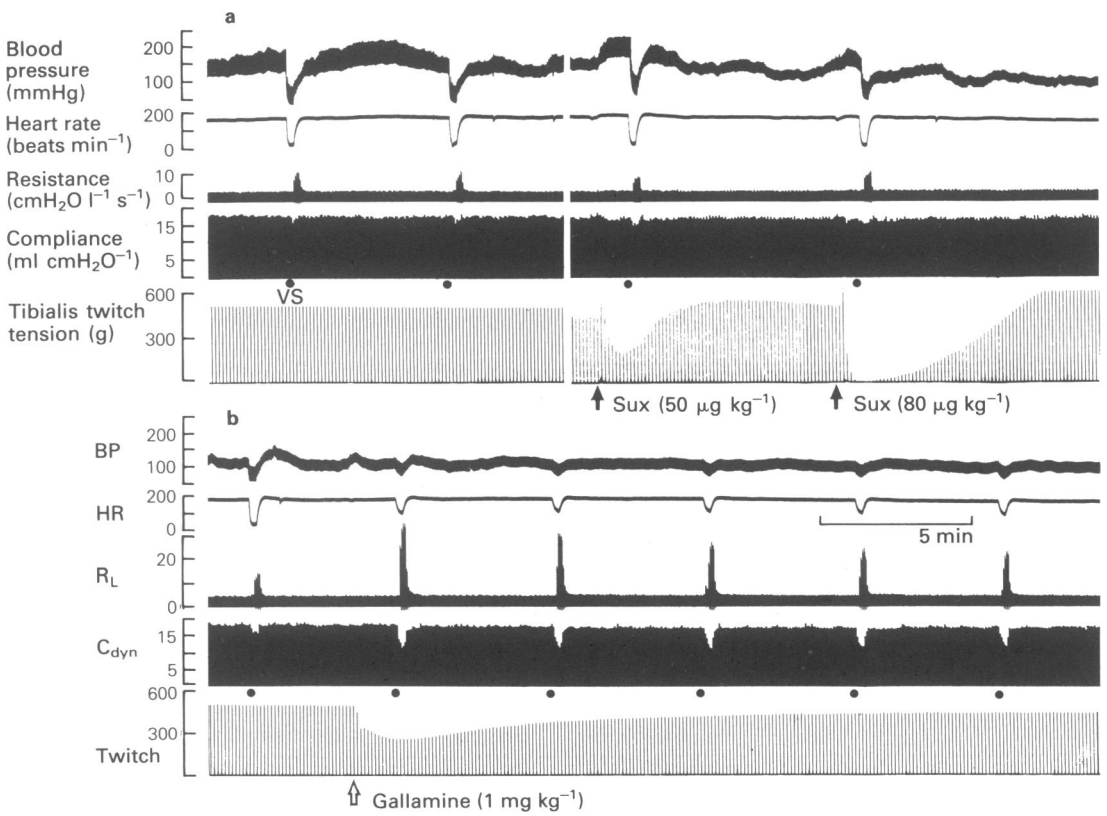

Figure 2 Stimulation of the vagus nerve (VS) at 10 V, 0.2 ms, 300 pulses per train produced bronchoconstriction shown as an increase in R_L and a decrease in C_{dyn} together with a fall in heart rate (HR) and blood pressure (BP). Suxamethonium paralyzed indirectly elicited twitches of the tibialis anterior muscle (5 V, 0.1 ms, 0.1 Hz) but had no effect on the pulmonary or cardiovascular responses to VS (section a). Gallamine (1 mg kg^{-1}) also paralyzed neuromuscular transmission but potentiated the increase in R_L and decrease in C_{dyn}, and inhibited the fall in heart rate produced by VS (section b).

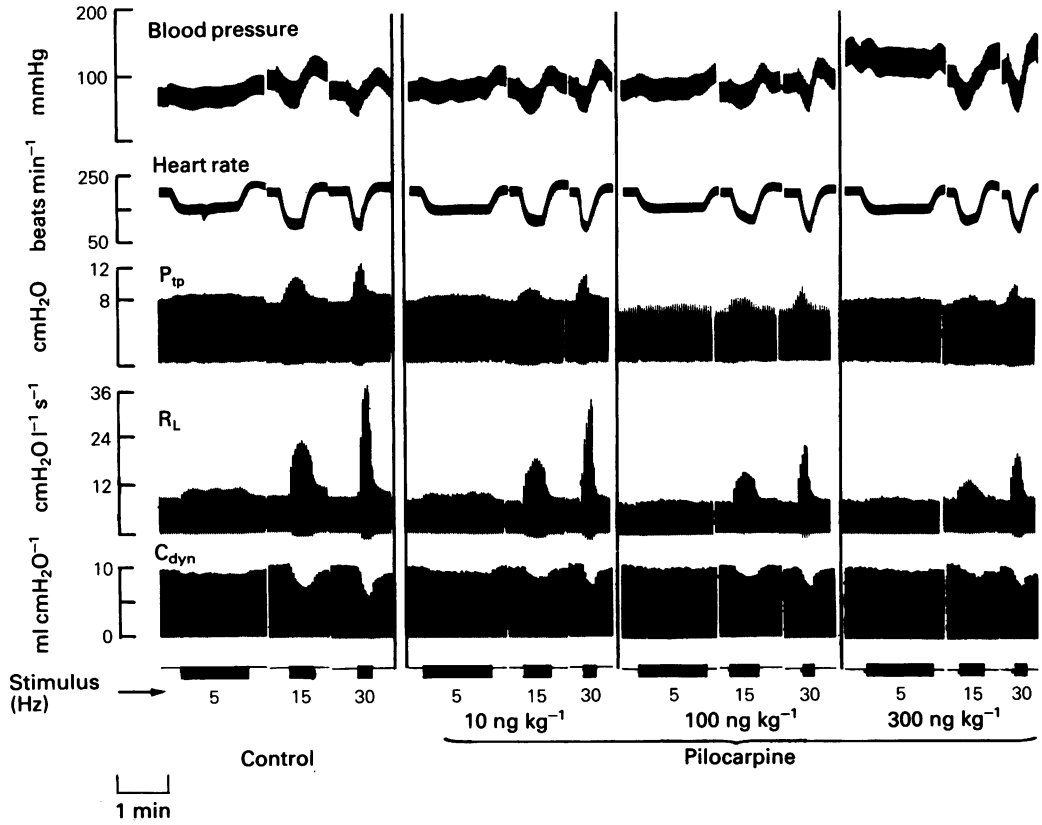

Figure 3 Effects of cumulative doses of pilocarpine on vagally-induced bronchoconstriction in an anaesthetized cat. Measurements of blood pressure, heart rate (beats min^{-1}), transpulmonary pressure (P_{tp}), lung resistance (R_L), and dynamic lung compliance (C_{dyn}) are shown. The left hand section shows that stimulation of the vagus nerve (10 V, 0.2 ms, 300 pulses per train) produced frequency-related bronchoconstriction recorded as an increase in P_{tp} and R_L and a fall in C_{dyn}. Cumulative administration of pilocarpine, in doses that did not alter the baseline R_L or C_{dyn}, inhibited vagally-induced bronchoconstriction. This effect was dose-related.

In contrast to the potentiating effect on vagally-induced bronchoconstriction, gallamine reduced ACh-induced bronchoconstriction. This depressant effect was not related to the dose of gallamine but was observed in every animal and is attributed to an action of gallamine on muscarinic receptors in airway smooth muscle.

Gallamine reduced both vagally-mediated and ACh-induced bradycardia. The bradycardia was always accompanied by a fall in blood pressure, which is not shown in the figure. Therefore, the potentiating effect of gallamine on vagal nerve stimulation in the lung was not related to cardiovascular changes associated with its ability to abolish vagally-mediated bradycardia.

In these experiments constriction of airway smooth muscle was also produced by intravenous injection of 5-hydroxytryptamine (5-HT) which does not act via muscarinic receptors. As the cats were vagotomised, reflex responses to 5-HT were also excluded. Gallamine had no effect on 5-HT-induced bronchoconstriction.

A comparison of the effect of gallamine on the heart, lung and neuromuscular junction is seen in Figure 2b. Injection of gallamine (1 mg kg^{-1}, i.v.) potentiated vagally-mediated bronchoconstriction, reduced vagally-mediated bradycardia and partially paralyzed neuromuscular transmission in the tibialis muscle. Thus, a dose of gallamine which paralyzed neuromuscular transmission also affected neuronal muscarinic receptors in the lung and postsynaptic muscarinic receptors in the heart. Neuromuscular blocking doses of suxamethonium (Figure 2a) had no effect on cardiac or pulmonary vagally-induced responses, neither did (+)-tubocurarine (1 mg kg^{-1}, i.v.; not shown); therefore it is unlikely that suxamethonium depressed the responses to vagal stimulation during the control period.

The opposite effect of vagally-induced bronchoconstriction was obtained with the muscarinic receptor agonist, pilocarpine. Figure 3 shows that pilocarpine inhibited vagally-induced bronchoconstriction in doses ranging from 10 ng to 300ng kg^{-1}. At these doses pilocarpine lacked any bronchoconstrictor effects as the baseline Cdyn and RL are not changed. The inhibitory effect of pilocarpine on vagally-induced bronchoconstriction could be reversed by a subsequent injection of gallamine (3 mg kg^{-1}, i.v.).

Guanethidine (4 mg kg^{-1}, i.v.) was used to deplete neuronal catecholamines. Subsequent injection of gallamine potentiated vagally-mediated bronchoconstriction as before. At the end of these experiments, depletion of noradrenaline was confirmed by the absence of a pressor response to tyramine (10 mg kg^{-1}, i.v.).

Discussion

The existence of inhibitory, neuronal muscarinic receptors has previously been demonstrated in parasympathetic nerves supplying airway smooth muscle in the guinea-pig (Fryer & Maclagan, 1984). Gallamine is an antagonist and pilocarpine an agonist for these receptors so that these drugs produce potentiation or inhibition of vagally-induced bronchoconstriction respectively. The experiments described in this paper show that similar receptors can also be demonstrated in pulmonary parasympathetic nerves of the cat.

The method used in these experiments in the cat can discriminate between responses occurring in central and peripheral airways. The central airways are defined as those perfused by the bronchial arteries while the peripheral airways are perfused by the pulmonary circulation, and include the respiratory bronchioles and alveolar ducts (for references see Hahn & Nadel, 1981). It was found that gallamine potentiates both the compliance and resistance changes produced by vagal stimulation and as these measurements are considered to reflect changes in peripheral and central airways respectively (Widdicombe, 1963) our results indicate that inhibitory muscarinic receptors are present in the nerves innervating trachea, bronchi and respiratory bronchioles. It is known that the parasympathetic nerves innervate airway smooth muscle throughout the bronchial tree and that the density of innervation decreases as the smaller airways are reached (see review by Gross & Skorodin, 1984). The results with gallamine suggest that inhibitory muscarinic receptors are present in parasympathetic nerves innervating all conducting airways.

In this type of experiment, characterization of the receptors is complicated by the fact that muscarinic receptors are located both on the nerve and (postsynaptically) on airway smooth muscle. Drugs which have a potent postsynaptic effect cannot, therefore, be used, so most of the currently available muscarinic agonists and antagonists are unsuitable. However, two drugs have been found which distinguish between the neuronal and postsynaptic receptors. Gallamine is an antagonist for the neuronal receptor at doses which have little postsynaptic action and the agonist, pilocarpine, inhibits neuronal receptors at doses which are 50 to 100 times lower than the doses required to produce salivation (Langley, 1878), bradycardia or bronchoconstriction by an action on the postsynaptic receptors.

It seems likely that the muscarinic receptors in the pulmonary parasympathetic nerves are similar to other muscarinic receptors which have been reported in autonomic nerves. Gallamine has been shown to be an antagonist for muscarinic receptors on noradrenergic nerves to the heart (Libet & Tosaka, 1970), on dopaminergic interneurones (Gardier *et al.*, 1978) and on the soma of ganglion cells (Gardier *et al.*, 1974; Ashe & Yarosh, 1984) in the sympathetic ganglia. All of these receptors have an inhibitory function. The muscarinic receptors on the pulmonary parasympathetic nerves described in this paper would therefore appear to belong to the same category as the inhibitory receptors described in other autonomic nerves.

Although the existence of neuronal muscarinic receptors in the pulmonary vagal nerves has been demonstrated by pharmacological methods, it has yet to be established whether these inhibitory receptors are activated during reflex stimulation of the nerves. In this context, it is interesting to note that in many of the experiments carried out in the last 20 years to elucidate the reflex control of airway smooth muscle by the parasympathetic nervous system, gallamine was used as the neuromuscular blocking drug (Nadel & Widdicombe, 1962; Olsen *et al.*, 1965; Karczewski & Widdicombe, 1969; Mills *et al.*, 1969; Colbatch & Engel, 1974).

The anticholinoceptor bronchodilator drugs currently in clinical use are probably nonselective antagonists which block all classes of muscarinic receptors. If neuronal muscarinic receptors exist in man and are able to attenuate ACh output, then the 'ideal' anticholinoceptor bronchodilator drug should lack antagonist actions on the neuronal muscarinic receptors. This would exclude the possibility that blockade of the neuronal receptors might increase transmitter output to oppose the postsynaptic action of the drug.

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