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MODULATION OF BRONCHOCONSTRICTOR RESPONSES TO HISTAMINE IN PITHED GUINEA-PIGS BY SYMPATHETIC NERVE STIMULATION

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1 Electrical stimulation (40V, 0.5–8 Hz, pulse width 0.5 ms) of the thoracic spinal outflow for between 10 and 120 s inhibited histamine-induced bronchoconstriction in pithed guinea-pigs.

2 The degree of this bronchodilatation varied with the position of the stimulating electrode within the spinal canal. Two maxima were identified. The first, at the level of the 9th and 10th thoracic vertebrae, was abolished by adrenalectomy. The second, at the level of the 3rd and 4th thoracic vertebrae, was associated with tachycardia and was unchanged by adrenalectomy.

3 The magnitude of this second bronchodilator effect varied with the frequency of stimulation. It was abolished by pretreatment with reserpine (5 mg/kg i.p. 48 and 24 h beforehand) and was competitively blocked by propranolol (0.01–1.0 mg/kg).

4 These observations are consistent with the view that bronchodilator tone is derived from neuronally-released noradrenaline within the lung. The noradrenaline probably overflows from well-innervated vasculature adjacent to sparsely innervated airways.

Introduction

The autonomic control of bronchial smooth muscle tone, and thus airway calibre, has possible relevance to airway hyperreactivity in asthma. The excitatory (constrictor) innervation to this tissue has been defined clearly as cholinergic. However, opinion is divided about the exact nature of the opposing inhibitory (bronchodilator) innervation (Richardson, 1979). In guinea-pigs this inhibitory pathway was thought initially to be entirely adrenergic (Foster, 1964; McCulloch, Proctor & Rand, 1967). More recently, a separate non-adrenergic pathway in guinea-pigs has been described *in vivo* (Chesrown, Venugopalan, Gold & Drazen, 1980) and in studies using electrically stimulated isolated trachea (Coleman & Levy, 1974; Richardson & Bouchard 1975; Kalenberg & Satchell, 1979). Because the trachea is more densely innervated by adrenergic nerve fibres than the lower airways, (O'Donnell, Saar & Wood, 1978), the *in vitro* observations may have limited relevance to the modulation of bronchial smooth muscle tone in whole lungs. Of more importance is the observation by Burden, Parkes & Gardiner (1971) that stimulation of the thoracic sympathetic spinal outflow inhibits bronchoconstrictor responses in pithed guinea-pigs. However, in this study the complete thoracic spinal outflow was stimulated. Therefore, the bronchodilator effect of neurotransmitters released in the lungs may have been obscured by the concomitant release of adrenal catecholamines. For this reason we have reinvestigated the pharmacology of this bronchodilator effect by stimulating discrete regions of the spinal cord, thereby excluding the simultaneous activation of both neuronal and hormonal bronchodilator pathways.

Methods

Adult (400–600 g) Hartley strain, male guinea-pigs were used throughout. Anaesthesia was induced with 5% halothane and the trachea cannulated with polyethylene tubing passed down to the level of the carina. A pithing rod (16 gauge oral dosing needle) was then inserted into the skull via an incision in the cranium, and advanced down the entire length of the spinal canal. The pithing rod incorporated within its length a miniature electrode assembly (Armstrong & Boura, 1973). The animals were immediately connected via the tracheal cannula to a small animal respirator (Searle Bioscience) delivering 52 strokes per min of 1 cc of laboratory air per 100 g body weight. At this stage some animals were adrenalectomized through bilateral posterior subcostal inci-

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sions, subsequently closed by sutures. Changes in pulmonary inflation pressure were measured by means of a transducer (Statham P23AA or Elcomatic EM750) in the tracheal circuit, a modification of the method originally described by Dixon & Brodie (1903). The right common carotid artery was cannulated to allow measurement of blood pressure (Statham P23Db or Elcomatic EM750 transducer connected to a Beckman coupler). Heart rate was monitored by means of a tachometer (Beckman) triggered by the arterial pulse wave. All of these parameters were recorded on a Beckman Type R Dynograph. Typical resting values were, pulmonary inflation pressure 12 mmHg; arterial blood pressure 50/35 mmHg; heart rate 270 beats/min. Drugs were injected through cannulae inserted caudally into the external jugular veins. Body temperature (monitored by rectal probe) was maintained at 37°C by heat from an infra-red lamp.

Standardization of bronchoconstrictor responses

Reproducible bronchoconstrictor responses of from 10 to 20 mmHg were obtained by injecting histamine (2 to 20 μg/kg) intravenously at 5 min intervals. Responses of this magnitude represented 30 to 60% of the maximum possible bronchoconstriction and were usually accompanied by transient hypertension and tachycardia.

Electrical stimulation of spinal nerve trunks

The technique used followed that of Burden *et al.* (1971) modified for the guinea-pig from the original method of Gillespie & Muir (1967), with the important exception that discrete areas of the thoracic spinal outflow rather than its entirety were stimulated (Gillespie, McLaren & Pollock, 1970). Stimulation parameters were 40V, pulse width 0.5 ms, frequency 0.125 to 8 Hz. Unless otherwise indicated the period of stimulation was 120 s beginning 90 s before an intravenous injection of histamine. Skeletal muscle twitches accompanying stimulation were blocked by tubocurarine (1 mg/kg i.v.) injected initially and thereafter at intervals of between 90 and 120 min.

Reserpine pretreatment

Randomly selected animals were pretreated with reserpine (5 mg/kg i.p.) 48 and 24 h prior to being prepared for experiments and then immediately adrenalectomized. Depletion of transmitter noradrenaline by this treatment regime was confirmed by

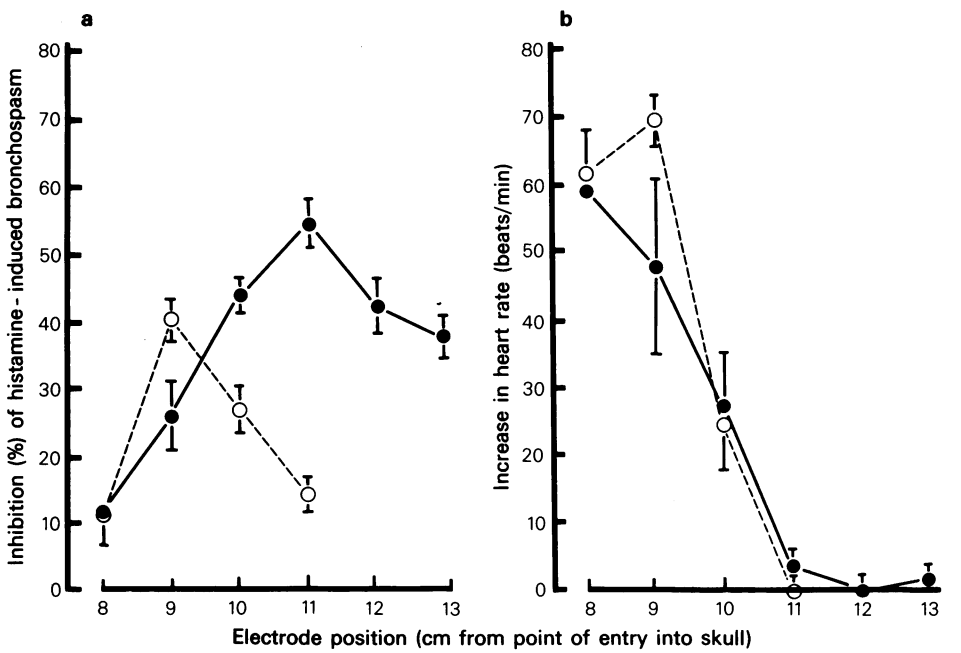

Figure 1 (a) Inhibition of histamine-induced bronchospasm (bronchodilatation) together with (b) concomitant increases in basal heart rate following electrical stimulation (1 Hz for 120 s) of the spinal nerve trunks of pithed guinea-pigs with the stimulating electrode at various positions within the thoracic spinal canal. (●) Intact animals, $n = 3-7$; (○) adrenalectomised animals, $n = 5$. Vertical bars, indicating s.e.mean, are shown where $n > 3$. The 9 cm and 11 cm positions correspond to thoracic vertebrae 3/4 and 9/10 respectively.

the absence of a pressor response to intravenous tyramine (10 mg/kg), a dose ten fold greater than that required to produce marked hypertension in control animals.

Materials

Drugs used were: halothane (May and Baker); histamine diphosphate, (±)-propranolol hydrochloride, reserpine and tyramine (Sigma); tubocurarine chloride (Burroughs Wellcome). All drugs given intravenously were dissolved initially and then diluted in sterile 0.85% saline. Reserpine was dissolved at a concentration of 36 mg/ml in a mixture of warm benzyl alcohol (0.2 ml) and Tween 80 (0.5 ml) and then diluted to 2.5 mg/ml with distilled water before intraperitoneal injection.

Results

Electrical stimulation (1 Hz) of the thoracic spinal outflow consistently inhibited histamine-induced bronchospasm. This inhibition will be defined as bronchodilatation throughout this study. The degree of bronchodilatation varied with the position of the stimulating electrode. In intact animals maximum bronchodilatation, unaccompanied by tachycardia, occurred when the electrode was approximately 11 cm from the point of entry of the pithing rod into the skull (Figure 1a). In contrast, after adrenalectomy maximum bronchodilatation occurred when the electrode was positioned more cranially (9 cm from point of entry). At this position marked tachycardia accompanied nerve stimulation both in intact and adrenalectomized animals (Figure 1b). The location of the electrodes, revealed by X-ray photography, showed that when positioned 9 and 11 cm from point of entry into the skull the electrode was stimulating spinal nerve roots associated with thoracic vertebrae 3 to 4 (T3) and 9 to 10 (T9) respectively. Bronchodilatation following stimulation at T9, which is anatomically close to the adrenal glands, was virtually abolished by adrenalectomy (Figure 1a). This selective bronchodilator effect can be attributed to release of adrenaline from the adrenal medulla. Accordingly, subsequent experiments were designed to investigate the pharmacology of bronchodilatation provoked by nerve stimulation at the more cranial position, T3. For this purpose stimulation parameters that produced just maximal bronchodilatation were sought by varying either the frequency or duration of nerve stimulation. In both intact and adrenalectomized animals bronchodilatation and tachycardia following stimulation at T3 for 120 s were related to frequency in the range 0.125 to 8 Hz, (Figure 2). When the duration of the stimulus at

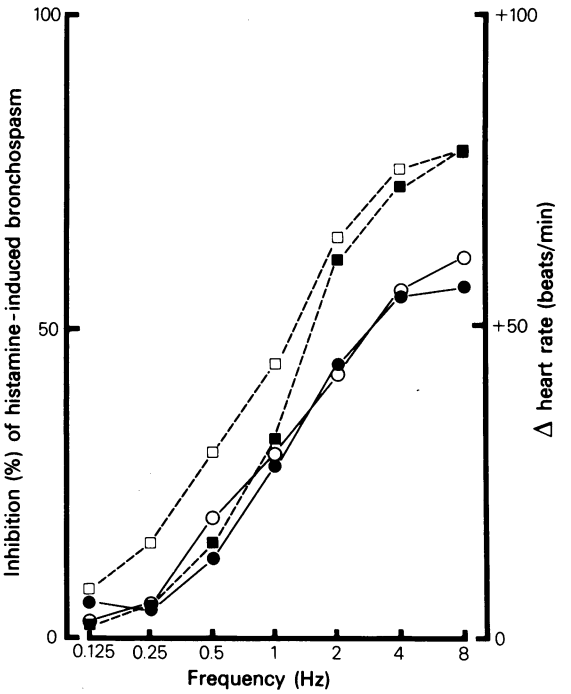

Figure 2 Frequency-response relationships for inhibition of histamine-induced bronchospasm (bronchodilatation) and elevation of basal heart rate following electrical stimulation (40 V, 120 s) of spinal nerve trunks of pithed guinea-pigs with the electrode in the T3 position. (●, ○) Airway responses in intact and adrenalectomized animals respectively; (■, □) corresponding changes in heart rate. Each point represents the mean result from experiments in 7 animals, standard errors of the mean have been omitted for clarity. The mean error for bronchodilatation was ±4.6% and for tachycardia ±8.6 beats/min.

T3 was reduced from 120 to 10 s ceasing immediately prior to the injection of histamine, the resulting bronchodilatation was essentially unchanged. The exception was at a frequency of 1 Hz when the shorter stimulus produced significantly less bronchodilatation. Persistence of the bronchodilator response was investigated by increasing the time interval between the beginning of a single stimulus train (8 Hz for 10 s) and the subsequent injection of histamine. Figure 3 illustrates that bronchodilatation declined as this interval was increased from 10 s to 4 min. Thus, stimulation at 8 Hz for 10 s, ceasing immediately before the injection of histamine, produced a just maximal bronchodilator response. Propranolol (0.01 to 1 mg/kg) strongly inhibited bronchodilatation by this stimulus. Inhibition was dose-related and accompanied by equivalent blockade of the concomitant tachycardia (Figure 4). Higher doses of propranolol

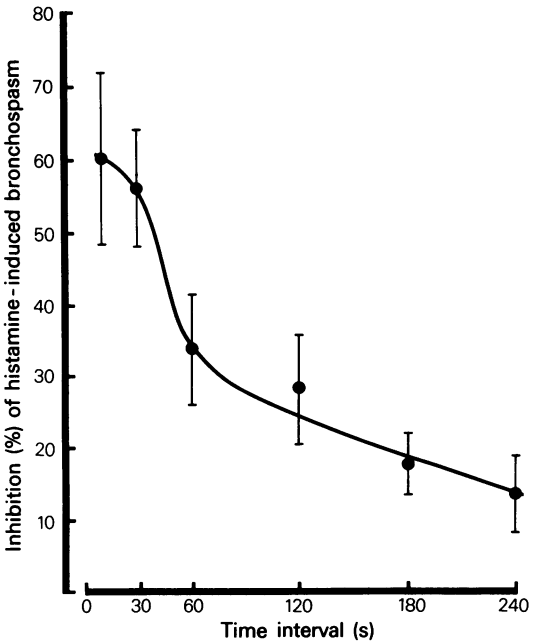

Figure 3 Inhibition of bronchospasm (bronchodilatation) in pithed guinea-pigs by electrical stimulation at T3 of the thoracic sympathetic outflow (40 V, 8 Hz for 10 s) for increasing time intervals (10 s–4 min) before injecting histamine. Each point represents the mean result from experiments in 5 animals; vertical lines show s.e.mean.

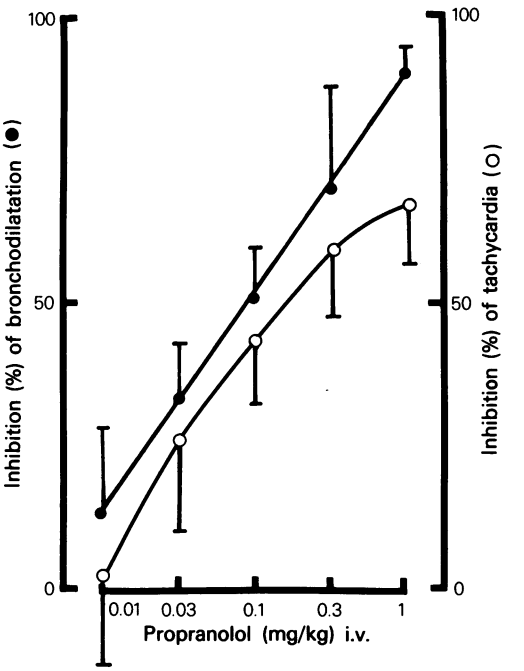

Figure 4 Inhibition by propranolol of both nerve stimulated (8 Hz, 10 s, T3) bronchodilatation (●) and concomitant tachycardia (○) in pithed guinea-pigs. Each point represents the mean ± s.e.mean (vertical bars) result from experiments in 7 to 12 animals in which the control value for inhibition of histamine-induced bronchospasm by nerve stimulation was 56.0 ± 4.1% (mean ± s.e.mean).

(1 to 3 mg/kg) were required to inhibit the bronchodilator effect of prolonged stimulation (120 s, 8 Hz) at T3. The tachycardia accompanying prolonged stimulation was also less well inhibited by propranolol (results not shown).

To investigate whether the bronchodilator effect of nerve stimulation at T3 was due to the release of neuronal noradrenaline, animals were pretreated with reserpine (see methods). The sensitivity to intravenous histamine was unchanged by this pretreatment. However, both tachycardia and bronchodilatation provoked by prolonged nerve stimulation (120 s, 8 Hz) at T3 were virtually abolished following reserpine.

Discussion

The present results confirm those of Burden *et al.* (1971) that electrical stimulation of the thoracic spinal outflow inhibits bronchoconstriction in pithed guinea-pigs. Two discrete bronchodilator pathways have been identified. One, originating at the level of the 9th and 10th thoracic vertebrae (T9), was clearly due to the release of adrenal catecholamines, and was absent in adrenalectomized animals. The released catecholamines (predominantly adrenaline) were bronchoselective, marked bronchodilatation being achieved without any concomitant tachycardia. The bronchodilator response following stimulation of the 3rd and 4th thoracic vertebrae (T3), which persisted after adrenalectomy, was accompanied by tachycardia and both responses were frequency-related. Bronchodilatation was relatively transient, becoming less as the interval between stimulation and the subsequent injection of histamine was increased. When just-maximal stimuli (8 Hz, 10 s) were used, propranolol blocked both bronchodilatation and tachycardia. There was no evidence that bronchodilatation was blocked less effectively than tachycardia. Higher doses of propranolol were required to inhibit both tachycardia and bronchodilatation when prolonged trains of stimuli were applied, indicating the competitive nature of such blockade. Reserpine pretreatment virtually abolished both the tachycardia and the bronchodilator responses to prolonged (8 Hz, 120 s) stimulation. This is clear evidence that the bronchodilator effect of nerve-

stimulation at T3 resulted from sympathetic nerve stimulation only.

Histological evidence shows that the adrenergic innervation to bronchial smooth muscle in the guinea-pig is very sparse (O'Donnell *et al.*, 1978). Thus, bronchodilatation probably results from the overflow of noradrenaline from the adjacent, well-innervated, vasculature. Direct measurements show that sympathetic nerve stimulation releases noradrenaline from perfused rabbit lung preparations (Tong, Mathe & Tisher, 1978). Since the bronchial smooth muscle of this species is also virtually devoid of sympathetic nerves (Mann, 1971), bronchial smooth muscle tone appears to be modulated *in vivo* by an adrenergic mechanism distinct from that of either circulating adrenaline or direct innervation. This contention is supported by previous studies showing that β-adrenoceptor blockade consistently enhances *in vivo* responses to bronchoconstrictor agents, both in normal and adrenalectomized animals (Collier, James & Piper, 1965; Farmer & Lehrer, 1966; McCulloch *et al.*, 1967).

Obviously it is not possible to carry out, in man, nerve stimulation experiments analogous to those described in this paper. Thus, conclusions about the nature of human bronchodilator pathways must be drawn from less direct evidence. Limited histochemical studies of human isolated airway smooth muscle have revealed no evidence for an adrenergic innervation of this tissue (Richardson & Beland, 1976). However, it is generally accepted that human lungs as a whole are innervated by sympathetic nerve fibres, the terminals of which are probably situated predominantly in the vasculature (Murray, 1976). In addition, non-selective β-adrenoceptor blocking drugs such as propranolol provoke bronchospasm, particularly in asthmatics (McNeill & Ingram, 1966). Despite recent conjecture about an alternative mechanism (Maclagan & Ney, 1979) this effect is best attributed to blockade of β-adrenoceptors in airway smooth muscle, a view derived from the study by Beumer (1967) who showed that impaired airway function was not associated with the dextrorotatory isomer of propranolol. Thus, the possibility exists that in man, as well as in the guinea-pig, sympathetic innervation to the pulmonary vasculature exerts bronchodilator tone over adjacent airway smooth muscle.

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