J. Pharm. Pharmacol. 1993, 45: 955–958
Received December 21, 1992

Effect of an Histaminergic H_3 Agonist on the Non-adrenergic Non-cholinergic Contraction in Guinea-pig Perfused Bronchioles

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Abstract—From the bronchioles of guinea-pigs, preparations were isolated for registration of perfused pressure on electrical field stimulation (EFS) and by application of drugs. The perfused bronchioles contracted when EFS was applied in the presence of atropine and phentolamine suggesting a non-adrenergic non-cholinergic (NANC) response. (*R*)-α-Methylhistamine (methylhistamine), a selective H_3 agonist, reduced the NANC bronchoconstrictor response in a concentration-dependent manner. β-Adrenoceptors, muscarinic and histamine (H_1 and H_2 receptor) antagonists, epithelial removal and cyclo-oxygenase inhibition had no effect on this inhibitory action of methylhistamine whereas the H_3 antagonist, thioperamide, reduced the inhibitory effect of methylhistamine with a K_i value of $2{\cdot}98 \times 10^{-9}$ M. Methylhistamine had no effect on the concentration-dependent contraction induced by exogenous substance P and neurokinin A, demonstrating that an H_3 receptor might inhibit the release of transmitter from NANC nerves on guinea-pig perfused bronchioles in-vitro.

Resting tone in airway smooth muscle is controlled by the autonomic nervous system, which includes excitatory and inhibitory nerves (Grundstrom et al 1981). There is an important relation between inflammation and neural control, so that several mediators may modify the release of neurotransmitters from airway nerves (Leff 1988).

Using the histamine H_3-receptor agonist, (*R*)-α-methylhistamine (methylhistamine), H_3 receptors have been shown to be involved in the feedback control of both histamine synthesis and release (Arrang et al 1983, 1987, 1988). Ishikawa & Sperelakis (1987) reported that histamine depresses sympathetic neurotransmission on perivascular nerve terminals by interacting with H_3-receptors while it has also been shown that an H_3 agonist modulates cholinergic and non-adrenergic non-cholinergic (NANC) neurotransmission in guinea-pig and human airway tissue (Ichinose & Barnes 1989a, b; Ichinose et al 1989). Up to now, there has been no indication of whether this third class of histamine receptor modulates the NANC bronchoconstriction induced by electrical stimulation on guinea-pig airways other than in-vivo.

The aims of this study were to define electrical field stimulation variables that result in nerve stimulation of the guinea-pig perfused bronchioles (Burgaud et al 1992), to characterize the type of innervation that is activated by field stimulation, and to investigate whether methylhistamine influences this response.

Materials and Methods

Preparation of guinea-pig perfused bronchioles

Hartley guinea-pigs of either sex, 400–500 g, were anaesthetized with urethane ($1{\cdot}5$ g kg^{-1}, i.p.) and bled. The lung was quickly removed and placed in modified Krebs–Henseleit solution (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·6, KH_2PO_4 1·2, $NaHCO_3$ 24·9 and glucose 11, gassed with 5% CO_2–95% O_2, pH 7·3–7·45.

A bronchiolar segment of the pulmonary cardiac lobe (0·3 mm i.d. about 3 mm long) was cannulated with a short, polished hypodermic needle (0·4 mm o.d.). The cannulated bronchiole was placed in an organ bath at 37°C and perfused at a constant rate of 1·0 mL min^{-1} using a peristaltic pump. Each bronchiole was placed between parallel platinum electrodes ($45 \times 7 \times 0{\cdot}1$ mm) in a 100 mL jacketed chamber and equilibrated for 60 min. The muscle was subjected to an electrical stimulus when the baseline pressure had stabilized. The increase of pressure generated in response to the stimulus was measured.

Response of airway preparations to electrical field stimulation (EFS)

Square-wave electrical impulses were delivered through the platinum electrodes in the bath using a direct-current power supply triggered by a stimulation. The effect of altering stimulus variables was studied by changing the stimulus over a range of voltages (10, 20, 40, 60 V) and a range of pulse durations (0–2·25 ms) at a frequency of 20 Hz. When a maximal voltage-pulse duration was determined, the perfused bronchioles were stimulated at 20 V and 2 ms over a range of frequencies (10–60 Hz). All values were interpreted as a percentage of 10^{-4} M acetylcholine-induced maximal contraction.

The effect of 2×10^{-5} M atropine, 2×10^{-5} M phentolamine, 5×10^{-7} M tetrodotoxin or 10^{-3} M cocaine on the response of guinea-pig bronchioles to the EFS was studied to determine whether the contractile response was attributable to the activation of the NANC nerves. Concentrations of all drugs were expressed as total molar concentration in the bath.

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Protocol

All experiments were performed in the presence of atropine (2×10^{-5} M) and phentolamine (2×10^{-5} M).

When we tested the effect of methylhistamine on EFS-induced contraction, tissues were incubated for 10 min with increasing concentration of H_3 agonist (2×10^{-14}–2×10^{-7} M). Parallel experiments were carried out in the presence of mepyramine (10^{-5} M) and cimetidine (10^{-5} M), propranolol (10^{-6} M), indomethacin (cyclo-oxygenase inhibitor) (10^{-5} M) or thioperamide (10^{-7}–3×10^{-4} M).

To determine whether guinea-pig airway epithelium possesses the ability to decrease the effect of methylhistamine, we removed epithelium by gently rubbing the luminal surface with a pipe cleaner before cannulation.

Cumulative concentration-response curves for substance P (2×10^{-10}–2×10^{-7} M) or neurokinin A (10^{-10}–5×10^{-8} M) were recorded by addition of these substances to the tissue bath. To determine whether methylhistamine modified contraction induced by substance P or neurokinin A, we introduced the H_3 agonist (2×10^{-7} M) in the organ bath 30 min before the agonist.

Drugs

The drugs used were: acetylcholine chloride, atropine, phentolamine, substance P, neurokinin A, tetrodotoxin, cocaine, indomethacin (Sigma, USA), cimetidine, mepyramine (SmithKline Beecham), methylhistamine, thioperamide (Bioprojet, France). Methylhistamine and tetrodotoxin were diluted in water and stored as 100 mL portions at −20°C and used as required. Thioperamide was diluted in dimethylsulphoxide and stored at 0–5°C. Indomethacin was dissolved in ethanol. Substance P and neurokinin A were dissolved in 0·9% NaCl.

Data analysis

Data were expressed as means ± s.e.m. Frequency, pulse duration, voltage, substance P and neurokinin A response curves were expressed as a percentage of the response to 10^{-4} M acetylcholine (78·2 ± 12·9 mmHg) (n = 45). When methylhistamine was tested alone or in the presence of antagonists, results were expressed as a percentage of inhibition of maximal contraction.

Values of median effective concentration (EC50) were established from the concentration-response curves plotted for each agonist in each segment. Data were analysed using Student's *t*-test for unpaired data. Probability values of $P < 0.05$ were considered significant.

The apparent dissociation constant (K_i) of thioperamide, added at fixed concentration to methylhistamine in increasing concentrations, was calculated according to Cheng & Prusoff (1973).

Results

The response of guinea-pig perfused bronchioles to EFS was rapid and reached a maximum within 20 s. The EFS was terminated when the maximum amount of contraction was obtained. The contractile response of perfused bronchioles to EFS depended on the voltage, pulse duration and frequency of the stimulus (Fig. 1). The minimal electrical stimulation variables necessary to obtain a maximal response were 20 V, 50 Hz and 2 ms.

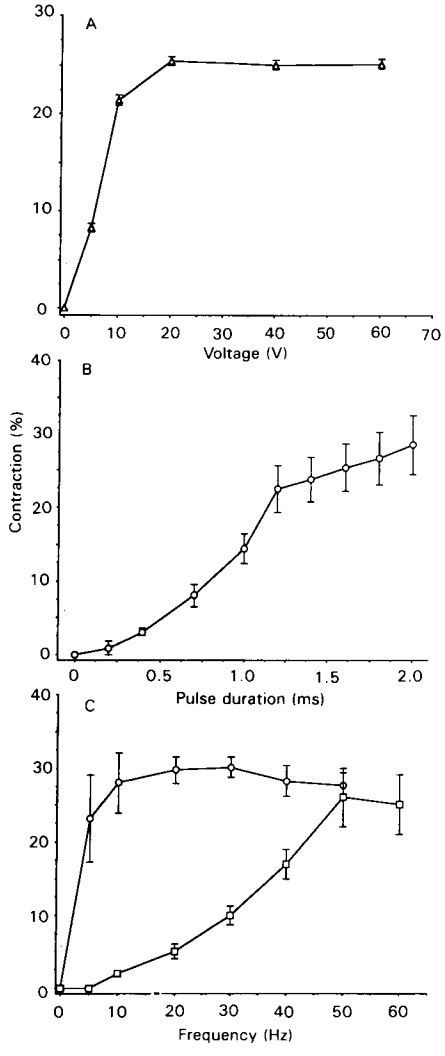

FIG. 1. Contractile response of perfused bronchioles to electrical field stimulation. A. Effect of voltage, 20 Hz, 0·5 ms (n = 6). B. Effect of pulse duration, 20 V, 20 Hz (n = 14). C. Effect of frequency, 20 V, 2 ms, with (□) and without (○) epithelium (n = 6). Contraction was expressed as a percentage of the response to 10^{-4} M acetylcholine (see data analysis).

The contractile response was completely abolished by tetrodotoxin (5×10^{-7} M) or cocaine (10^{-3} M), while atropine (2×10^{-5} M) or phentolamine (2×10^{-5} M) failed to prevent this contraction (Fig. 2).

Methylhistamine (2×10^{-14}–2×10^{-7} M) gave a concentration-dependent inhibitory effect on EFS-induced contraction. The EC50 value for this effect was $1{\cdot}3 \pm 0{\cdot}4 \times 10^{-10}$ M and the maximum inhibitory effect was 94·2 ± 1·8% at 2×10^{-7} M (n = 7) (Fig. 3).

Mepyramine (10^{-5} M), cimetidine (10^{-5} M) or propranolol (10^{-6} M), to block H_1-, H_2-histaminergic receptors or β-adrenoceptors, respectively, failed to influence the inhibitory effect of 2×10^{-7} M methylhistamine (Table 1). The selective H_3 antagonist, thioperamide (10^{-8}–3×10^{-4} M) inhibited the effect of methylhistamine in a concentration-dependent manner (Fig. 4) leading to a K_i value of $2{\cdot}98 \times 10^{-9}$ M.

On the other hand, epithelium removal shifted the frequency contraction curve to the left (Fig. 1) without

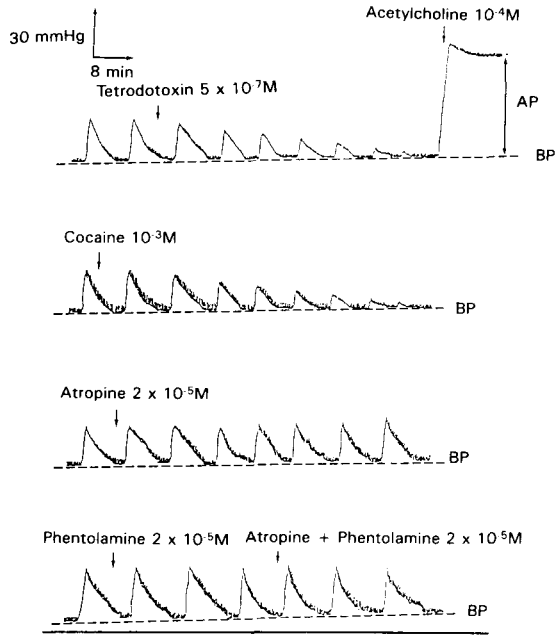

FIG. 2. Responses of guinea-pig perfused bronchioles on electrical field stimulation (20 V, 2 ms, 50 Hz) and application of drugs. Traces are redrawn from originals.

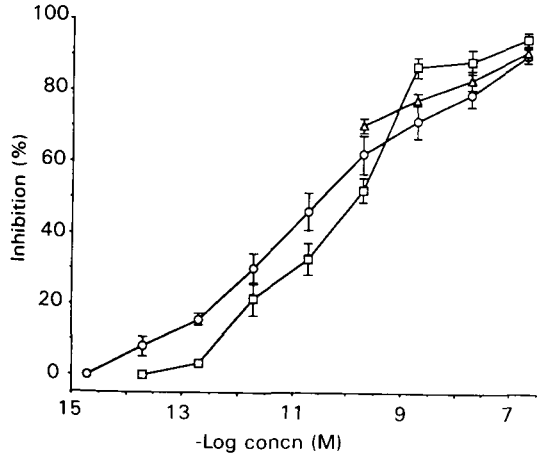

FIG. 3. Effect of methylhistamine on electrical field stimulation alone (□) (n = 7), in the presence of 10^{-5} M indomethacin (△) (n = 6) or without epithelium (○) (n = 7). Values are means with s.e.m. shown by vertical bars.

Table 1. The effect of blocking agents on the inhibition of NANC responses by methylhistamine (2×10^{-7} M).

Drugs	Inhibition (%)
Methylhistamine 2×10^{-7} M	94·2 ± 1·8
+ thioperamide 3×10^{-4} M	8·0 ± 0·5*
+ cimetidine 10^{-5} M and mepyramine 10^{-5} M	91·8 ± 2·5
+ propranolol 10^{-6} M	93·0 ± 1·4

* $P < 0.0001$ compared with the effect of methylhistamine alone. (n = 6).

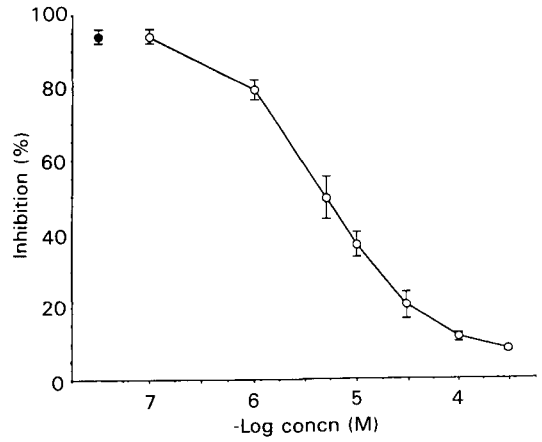

FIG. 4. Effect of thioperamide on the inhibition by 2×10^{-7} M methylhistamine of the NANC contraction (○) (n = 6). The closed symbol (●) represents the effect of 2×10^{-7} M alone (n = 7). Values are means with s.e.m. shown by vertical bars.

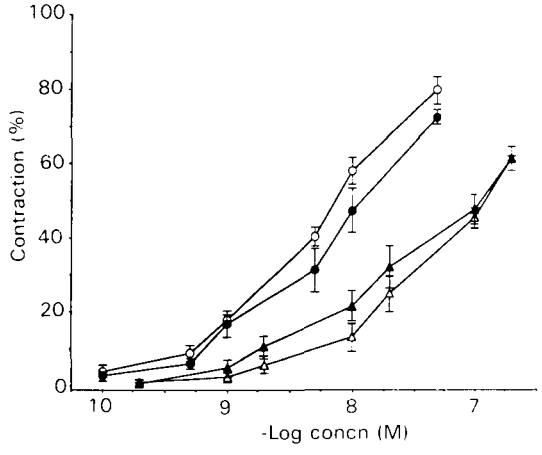

FIG. 5. Contractile concentration-response curves to neurokinin A (○, n = 7; ●, n = 6) and substance P (△, n = 7; ▲, n = 6) obtained on guinea-pig perfused bronchioles with (closed symbols) or without 2×10^{-7} M methylhistamine (open symbols). The substances were added cumulatively and the responses are expressed as a percentage of a maximum contraction to acetylcholine (10^{-4} M). Mean results are shown and vertical lines indicate s.e.m.

modifying the maximal effect (28·7 ± 2·9 and 25·4 ± 3·9 mmHg with or without epithelium, respectively ($P > 0.4$)).

The removal of epithelial cells or the addition of 10^{-5} M indomethacin did not modify the methylhistamine-induced inhibition of the EFS-induced contraction (Fig. 3).

Fig. 5 shows the concentration-dependent contractions induced by exogenous substance P (2×10^{-10}–2×10^{-7} M) or neurokinin A (10^{-10}–5×10^{-8} M). The presence in the bath of 2×10^{-7} M methylhistamine did not modify the effect of neuropeptides on the smooth muscle preparation ($P > 0.5$).

Discussion

EFS induced a bronchoconstriction which depended on the voltage, frequency and pulse duration of the stimulus applied. Exogenous substance P and neurokinin A, principal mediators of the NANC nervous system (Barnes 1986), induced a concentration-dependent bronchoconstriction.

Blockade of the contractile response by tetrodotoxin, a sodium channel blocker (Evans 1969), or cocaine but not by atropine or phentolamine indicated that the contractile response was linked to NANC nerves. Tetrodotoxin prevented the release of neuromediators from nerves but had no effect on the ability of smooth muscle to respond to exogenous mediators. This observation was supported by our finding that tetrodotoxin failed to alter the response of guinea-pig perfused bronchioles to 10^{-4} M acetylcholine (Kao 1966). An externally applied EFS induced a contraction by release of neurotransmitter at synapses and at the neuromuscular junction of the NANC system.

The aim of these experiments was to establish whether methylhistamine inhibited the release of mediators of the NANC nervous system in-vitro as in-vivo (Ichinose & Barnes 1989b). We demonstrated that methylhistamine caused a concentration-dependent inhibition of guinea-pig perfused bronchioles contraction induced by an EFS in the presence of atropinic- and α-adrenergic blockers. It will be noted that the H_3 agonist was more effective at inhibiting the NANC contraction in-vitro compared with NANC responses in-vivo.

The inhibitory effect of methylhistamine was blocked by thioperamide, an H_3-selective antagonist, with a similar potency to that seen in rabbit middle cerebral artery (Ea-Kim et al 1992) or to that observed for acetylcholine inhibition release in guinea-pig airways (Ichinose et al 1989). H_1 and H_2 antagonists failed to modify the effect of the H_3 agonist confirming that this effect was mediated via H_3 receptors in the NANC nervous system.

As methylhistamine (2×10^{-7} M) did not modify the concentration-dependent bronchoconstriction induced by exogenous substance P and neurokinin A, we concluded that H_3 receptors could exist on sensory nerves and modulate the release of neuromediators.

It has been reported that NANC neurotransmission could be modulated by catecholamines in guinea-pig airways (Grundstrom & Anderson 1985; Anderson et al 1986). In our experiments neither phentolamine nor propranolol modified the inhibitory effect of methylhistamine, demonstrating that α- or β-adrenoceptor blockade did not influence the effect of the H_3 agonist.

In a previous study, we demonstrated that methylhistamine could induce a bronchodilatation on guinea-pig perfused bronchioles (unpublished). This effect was an epithelium-dependent relaxation via the release of metabolites of arachidonic acid. Thus, another possible mechanism of methylhistamine-induced inhibition is the release of bronchodilator prostaglandins by the epithelial wall; however, since epithelium removal or 10^{-5} M indomethacin did not alter the effect of the H_3 agonist, and direct substance P- and neurokinin A-induced contractions were not influenced by methylhistamine, this hypothesis is unlikely.

Our results are similar to previous data obtained by Ichinose in-vivo (Ichinose & Barnes 1989b), which suggest that H_3 receptors are present in the NANC nervous system on guinea-pig perfused bronchioles. The activation of these receptors by methylhistamine inhibits the NANC bronchoconstriction induced by an EFS. However, since the H_3 agonist did not alter the effect of substance P and neurokinin A, we have demonstrated that these receptors have a presynaptic localization.

Acknowledgements

The authors are grateful to Dr J. M. Arrang for his constructive comments during the preparation of this manuscript, and for the excellent technical assistance provided by J. Javellaud.

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