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REACTIVITY OF ISOLATED TRACHEA, BRONCHUS AND LUNG STRIP OF CATS TO CARBACHOL, 5-HYDROXYTRYPTAMINE AND HISTAMINE: EVIDENCE FOR THE EXISTENCE OF METHYSERGIDE-SENSITIVE RECEPTORS

NARESH CHAND

Department d'Anatomie et Physiologie animales, Faculté de Medecine veterinaire, Université de Montreal, C.P. 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6 and Department of Physiology, SUNY, Department of Physiology, 450 Clarkson Avenue, Brooklyn, New York 11203, U.S.A.

1 The reactivity was investigated of cat isolated tracheal chains, bronchial spirals and lung parenchymal strips to carbachol, 5-hydroxytryptamine (5-HT) and histamine.

2 Carbachol and 5-HT produced concentration-dependent contractions of all three tissues, responses to the former drug being selectively blocked by atropine and to the latter by methysergide.

3 Histamine failed to elicit a contractile response from the tracheal or bronchial muscle. However, it induced dose-related contractions of the lung strips, that were selectively blocked by mepyramine.

4 It is concluded that lung parenchymal strips of cats possess mepyramine-sensitive histamine H_1-receptors. Receptors for 5-HT, susceptible to methysergide, occur in the tracheobronchial smooth muscles as well as in lung strips of cats.

Introduction

Traditionally 5-hydroxytryptamine (5-HT) is considered to act on at least two distinct sites, often referred to as 'M'-neuronal and 'D'-muscular tryptamine receptors. Responses mediated by M-receptors are antagonized by morphine, cocaine and atropine, whereas D-receptor-mediated responses are blocked by methysergide, dibenamine, dibenzyline, lysergic acid diethylamide and 2-bromolysergic acid diethylamine (Gaddum & Picarelli, 1957).

Isolated tracheal and bronchial smooth muscle preparations obtained from cats contract on exposure to 5-HT (Brocklehurst, 1958; Statkov, 1969; Turker & Ercan, 1976; Chand & Eyre, 1977a,b). Surprisingly, Statkov (1969) demonstrated that atropine, morphine, lysergic acid diethylamide as well as dihydroergotamine failed to influence 5-HT-induced contractions of feline trachea and concluded that neither M- nor D-receptors were involved in the action of 5-HT in this tissue. The present study sought to characterize the reactivity of central (trachea and bronchi) and peripheral (lung parenchymal strips; Lulich, Mitchell & Sparrow, 1976) airway smooth muscles to carbachol, histamine and 5-HT and evidence is presented for the existence of methysergide-sensitive receptors in cat airways.

Methods

The methods of tissue collection, preparation of chains from the trachea and spiral strips of bronchi and lung parenchymal strips (LPS) as well as the recording of drug responses and their modification by antagonists were similar to those described earlier (Lulich *et al.*, 1976; Chand & Eyre, 1977a, b; Chand, 1979; Chand, DeRoth & Eyre, 1979). In brief, 15 adult cats weighing between 2 to 4 kg, of either sex, were killed by rapid intravenous injection of pentobarbitone sodium (40 mg/kg). Trachea and lungs were immediately dissected out and placed in oxygenated Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 1.9, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2 and glucose 11.0.

A pair of tracheal chains, each consisting of 4 to 5 rings (Castillo & DeBeer, 1947), of bronchial spiral strips (2 to 4 mm diameter) (Chand & Eyre, 1977a) and of LPS (about 3 × 2 × 30 mm) (Lulich *et al.*, 1976; Drazen & Schneider, 1978) were prepared from each animal and mounted in separate isolated tissue baths containing Krebs-Henseleit solution continuously aerated with a 5% CO_2 plus 95% O_2 mixture, at 37°C. The tissues were allowed to stabilize for at least 2 h under a resting load of 2 g for the LPS and the

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Table 1 Comparative sensitivity of isolated trachea, bronchi and lung strips of the cats to carbachol, 5-hydroxytryptamine (5-HT) and histamine

Spamogen	n	*Minimum effective concentration* (M)	EC_{50} s
Trachea			
Carbachol	15	$9.5 \pm 4.2 \times 10^{-9}$	$3.1 \pm 1.4 \times 10^{-7}$
5-HT	15	$8.2 \pm 3.9 \times 10^{-8}$	$5.3 \pm 1.5 \times 10^{-7}$
Histamine	5	Inactive	Inactive
Bronchi			
Carbachol	8	$5.6 \pm 2.5 \times 10^{-8}$	$4.5 \pm 1.1 \times 10^{-6}$
5-HT	8	$7.1 \pm 3.3 \times 10^{-7}$	$5.4 \pm 1.7 \times 10^{-6}$
Histamine	5	Inactive	Inactive
Lung strips			
Carbachol	12	$9.1 \pm 1.6 \times 10^{-7}$	$4.2 \pm 2.4 \times 10^{-5}$
5-HT	12	$7.5 \pm 2.3 \times 10^{-7}$	$4.5 \pm 2.2 \times 10^{-5}$
Histamine	12	$1.5 \pm 2.7 \times 10^{-7}$	$8.5 \pm 3.8 \times 10^{-6}$

Values are mean ± s.e. mean.

bronchi, and 3 g for the trachea. Contractions were measured with E & M Isotonic Myograph transducers connected to a Fisher Recordall series 5000 pen recorder via a Narco Physiolgraph (Chand & DeRoth, 1979).

After single or cumulative concentration-curves to agonists had been established, a predetermined concentration of an antagonist was added to one tissue of each pair. Thirty min later concentration-response curves were repeated in the presence of the antagonist. The second tissue of each pair served as a control to monitor any time-related change in the sensitivity of the tissues to agonists. The effectiveness and specificity of the antagonist were calculated by measuring the dose-ratio; i.e., the ratio of equiactive concentrations (EC_{50} s) of agonist in the presence and absence of antagonist (Gaddum, Hameed, Hathway & Stephens, 1955) and were statistically analysed by Student's paired *t* test. The means ± s.e. mean of the agonist responses were determined.

Drugs used in this study were histamine diphosphate, 5-hydroxytryptamine creatinine sulphate (serotonin: 5-HT), carbamylcholine chloride (carbachol), atrophine sulphate (Sigma Chemical Co., St. Louis, M.), methysergide hydrogen maleinate (Sandoz Canada Ltd, Montreal) and mepyramine maleate (Poulenc Ltd., Montreal, Quebec).

Drug concentrations are expressed as final molar (M) bath concentrations.

Results

The results summarized in Table 1 show the differential sensitivity of cat isolated trachea, bronchi and lung parenchymal strips (LPS) to carbachol, 5-HT and histamine. Carbachol and 5-HT both caused concentration-dependent contractile responses of all three preparations , the maximal response to 5-HT being 40% to 60% of the carbachol maximal responses of both trachea and bronchi. By contrast, histamine (10^{-8} to 10^{-3} M) failed to elicit responses from the trachea and bronchi but produced concentration-dependent contractions of the LPS. 5-HT and histamine induced approximately equal maximal responses whereas carbachol induced only 40 to 50% of the histamine maximal response of the LPS. During the course of each experiment, no time-related change was observed in the sensitivity of these tissues to repeated agonist exposures.

The interactions of atropine, methysergide and mepyramine with the agonists on these tissues are summarized in Table 2. Atropine (10^{-8} or 10^{-7} M) blocked the contractions induced by carbachol on the three tissues, although a smaller dose-ratio was apparent in LPS, without affecting the contractions caused by 5-HT or histamine.

Mepyramine (10^{-7} M) antagonized histamine-induced contractions on the LPS (dose-ratio = 11 ± 3, n = 8) but was without effect against 5-HT or carbachol in this tissue (Table 2).

Methysergide (5×10^{-8} or 1×10^{-7} M) selectively antagonized the responses to 5-HT of the trachea, bronchi and LPS. Furthermore, methysergide (10^{-7} to 10^{-6} M added to the tissues at the peak of a contraction to 5-HT of either bronchus or trachea produced complete reversal (n = 15) (Figure 1). However, methysergide did not alter the responses to histamine or carbachol of any tissue.

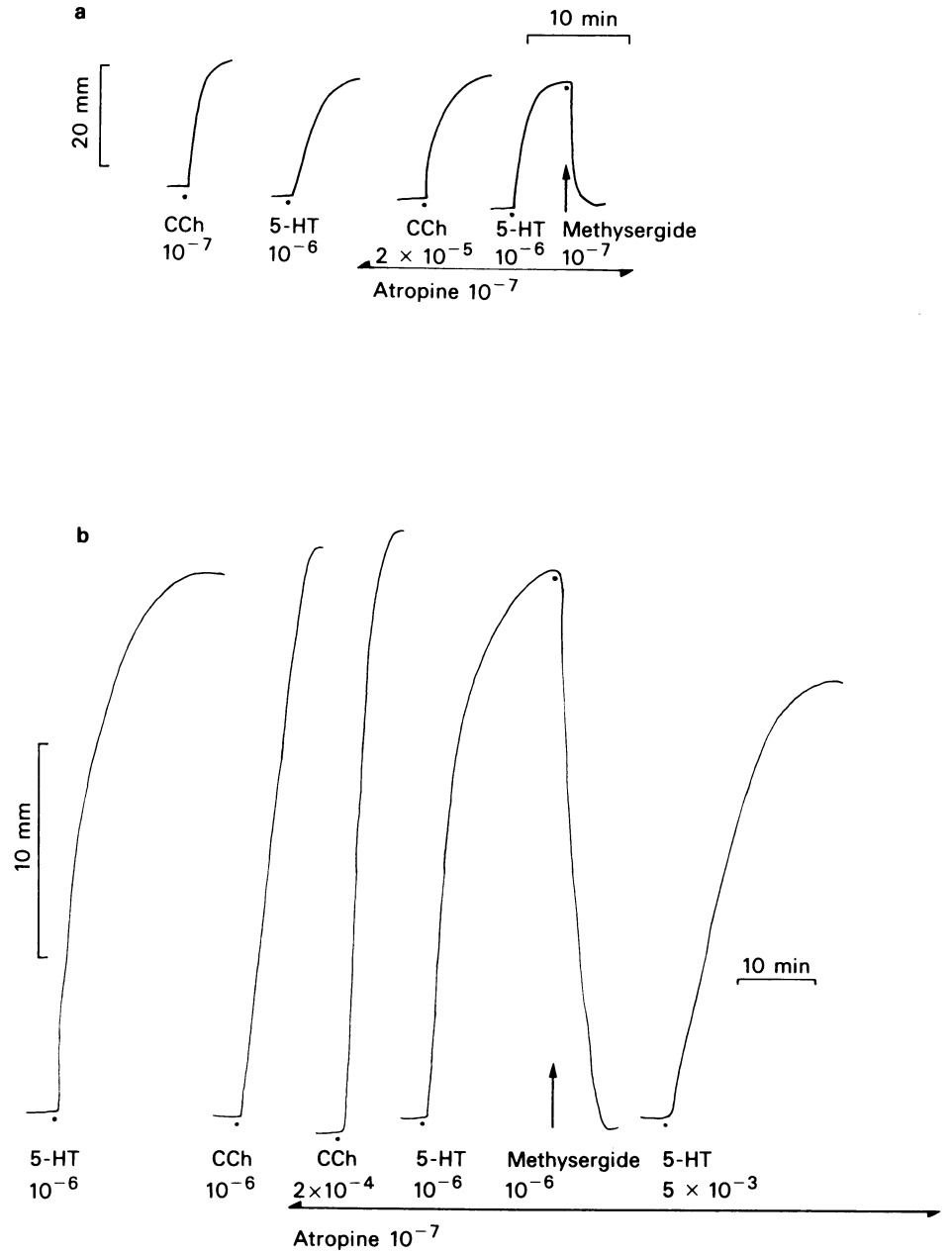

Figure 1 Failure of atropine to influence 5-hydroxytryptamine (5-HT)-induced contractions on cat isolated trachea (a) and bronchus (b), and their reversal by methysergide. Drug concentrations are molar (M). CCh = Carbachol.

Table 2 Dose-ratios of agonists in presence of antagonists in cat isolated trachea, bronchi and lung strips

Antagonists	*Bath conc.* (M)	*Dose-ratio*							
		Trachea		*Bronchi*		*Lung strips*			
		Carbachol	*5-HT*	*Carbachol*	*5-HT*	*Carbachol*	*5-HT*	*Histamine*
Atropine	10^{-8}	*42 ± 8 (5)	1 ± 0 (5)	*50 ± 5 (4)	1 ± 0 (4)	*5 ± 2 (3)	1 ± 0 (3)	1 ± 0 (3)
	10^{-7}	*120 ± 25 (8)	1.5 ± 0.5 (8)	*205 ± 30 (8)	1 ± 0 (8)	*15 ± 5 (4)	1 ± 0 (4)	1 ± 0 (3)
Methysergide	5×10^{-8}	1 ± 0 (5)	*500 ± 50 (5)	—	—	—	—	—
	10^{-7}	1 ± 0 (5)	*900 ± 98 (9)	1 ± 0 (4)	*1500 ± 200 (9)	1 ± 0 (4)	*15 ± 5 (6)	1 ± 0 (4)
Mepyramine	10^{-7}	—	—	—	—	1 ± 0 (5)	1 ± 0 (4)	11 ± 3 (8)

Values are mean ± s.e. mean. Numbers in the parentheses indicate the number of observations from different tissues.
* $P < 0.001$.

Discussion

The differential sensitivity of the cat isolated trachea, bronchus and lung parenchymal strips to carbachol, histamine and 5-HT, demonstrated in this study, is not inconsistent with similar findings in airway smooth muscles of several mammalian species (Brocklehurst, 1958; Persson & Ekman, 1976; Lulich *et al.*, 1976; Chand and Eyre, 1977a, b; Drazen & Schneider, 1978; Chand & DeRoth, 1979; Chand *et al.*, 1979; Chand, 1979; 1980; Chand & Altura, 1979). The marked differences in the reactivity of the central and peripheral airways to biogenic amines and carbachol implies that chemical mediators of immediate hypersensitivity may act differently according to the airways segment.

The antagonism of histamine-induced contractions of lung parenchymal strips by mepyramine demonstrated the existence of histamine H_1-receptors in feline peripheral airway smooth muscles; this is similar to the peripheral airways (lung strip) of dogs, horses, sheep and rabbits (Chand, 1980). Trachea and bronchus of cats and rhesus monkeys are usually unresponsive to histamine (Main, 1964; this study), or respond with relaxations to histamine, impromidine or dimaprit (H_2-agonists), when precontracted by carbachol (Turker & Ercan, 1976; Chand & Eyre, 1977a, b; Chand & Altura, 1979). These relaxations are antagonized by metiamide and cimetidine, showing the occurrence of inhibitory histamine H_2-receptors in the trachea of cats, terminal bronchus of sheep and tracheo-bronchial smooth muscles of rhesus monkeys (Chand & Altura, 1979; Chand, 1980). Thus, there are significant qualitative and quantitative regional differences in the reactivity of airway smooth muscles to histamine and in the distribution of histamine receptor subtypes, in closely related species i.e., in langur vs rhesus monkeys; cats vs dogs; cattle vs sheep (Chand, 1980). Therefore, extrapolation of observations from one species to another and even from one region of the airways to another within the same species is of doubtful merit.

In general, the constrictor effects of 5-HT on the airway smooth muscles may be mediated via both M (Offermeier & Ariens, 1966) and D tryptamine receptors (Mathe, Astrom & Persson, 1971). The failure of atropine (this study) and morphine (Statkov, 1969) to influence 5-HT-induced responses of the airway smooth muscles of cats makes it unlikely that 5-HT is acting indirectly by a release of acetylcholine through stimulation of M neuronal receptors. However, the antagonism or reversal of 5-HT-induced airway contractions by methysergide provides strong pharmacological evidence for the existence of D receptors in the airways smooth muscle. This observation counters the suggestion that cat trachea lacks both M and D-tryptamine receptors (Statkov, 1969).

Reprint requests to N.C. at Brooklyn address.

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