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The pharmacology of sheep tracheobronchial muscle: a relaxant effect of histamine on the isolated bronchi

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1. Histamine contracted the tracheal and major bronchial muscles of sheep and relaxed the muscles of the lesser bronchi and bronchioles.

2. The stimulant action of histamine on the trachea was antagonized by mepyramine, whereas the relaxant effect on the bronchial tree was not.

3. 5-Hydroxytryptamine contracted the musculature from all parts of the respiratory tract—an effect which was specifically antagonized by both methysergide and atropine.

4. Acetylcholine contracted all sheep tracheobronchial muscle and isoprenaline relaxed it.

5. Bradykinin had a very weak stimulant action.

6. It is concluded that histamine relaxes sheep bronchi by a direct excitation of histamine-sensitive receptors which are not blocked by mepyramine. No indirect components through autonomic nervous elements or by way of catecholamine release were evident. 5-Hydroxytryptamine seemed to have a dual mode of action in contracting sheep tracheobronchial muscle (*a*) directly on its own receptors and (*b*) indirectly through parasympathetic components.

The pharmacological actions of histamine, 5-hydroxytryptamine (5-HT) and other agents on the tracheobronchial musculature have been described in many species. In most animals histamine causes contraction of the airway muscle. Maengwyn-Davies (1968) has reported, however, that in the isolated tracheal muscle of the cat, histamine antagonized the contractions produced by carbachol; and by the use of antagonists concluded that histamine may have a dual mode of action on this preparation, namely combination with a "histamine receptor" and release of catecholamines. Alexander, Eyre, Head & Sanford (1967) reported that in live anaesthetized sheep, histamine caused increased airway resistance as measured by the method of Konsett & Rössler (1940).

Some interest has recently been focused on the anaphylactic response of ruminants (Aitken & Sanford, 1968; Alexander, Eyre, Head & Sanford, unpublished) and to

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assist the interpretation of data it seemed important to establish the "normal" pharmacological responses of tracheobronchial muscle of cattle and sheep, not only to histamine but to 5-hydroxytryptamine, acetylcholine, catecholamines and bradykinin, and their modification by known antagonists.

The present study describes the reactions of the isolated muscles of the respiratory airways of sheep.

Methods

Lung specimens were obtained from healthy lambs or yearling sheep of mixed breed and sex, slaughtered at the local abattoir. As soon as possible (usually 1 to 5 min) after shooting and "bleeding out" one whole lung was removed and immersed in cold Krebs-Henseleit (1932) solution, and transported to the laboratory with a delay normally less than 30 min.

Bronchial rings

Segments of bronchi were dissected out under cold Krebs and rings were cut from three "regions" of the respiratory tract: (1) "major" bronchi, within approximately 3 cm of the tracheal bifurcation; (2) "intermediate" bronchi, usually of approximately 0·5 cm diameter and having complete cartilages; (3) "terminal" bronchi with incomplete cartilages and of less than 0·4 cm. Six bronchial rings were joined together with thread, after the method described for guinea-pig trachea by Castillo & DeBeer (1947).

Tracheal muscle

The trachea of the sheep has a diameter of approximately 1·5–2·0 cm and possesses a comparatively stout musculature. The trachea was cut into individual cartilaginous rings from which the trachealis muscles were dissected. Two muscles were joined end to end with thread.

Bronchial or tracheal muscle preparations were set up in pairs in similar 20 ml. organ baths containing Krebs-Henseleit solution (1932) at 35° C, aerated with 95% oxygen and 5% carbon dioxide mixture.

The preparations were attached to frontal writing levers which exerted a moment of 3 *g* cm. In these conditions the muscle maintained partial tonus. Contractions were magnified 10 to 15 times and were recorded on a smoked kymograph drum.

Tissues were exposed to each agonist for 5 or 10 min every 30 min. Antagonists were applied when two consecutive doses of an agonist gave equal responses. The activity of the antagonists was measured by determining the ratio of doses of agonist which give equal responses in the presence and absence of antagonist. This is termed the dose ratio (Gaddum, Hameed, Hathaway & Stephens, 1955).

It was necessary to wait for 30–60 min after the response of each agonist for the preparation to return to the original base-line.

Drugs

The compounds used were acetylcholine chloride, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, isoprenaline hydrochloride, atropine sulphate, mepyramine maleate, methysergide bimaleate, propranolol hydrochloride,

phentolamine hydrochloride, bretylium tosylate, cocaine hydrochloride, thiopentone sodium, and pentobarbitone sodium. Concentrations referred to are expressed as the quantities of these salts per ml.

Results

Intermediate and terminal bronchi

Acetylcholine

Acetylcholine 2–5 μg/ml. caused a contraction of the muscle with a delay of 10–15 sec (Fig. 1). Atropine 0·1–1·0 μg/ml. specifically antagonized acetylcholine. Partial inhibition of acetylcholine by mepyramine 10 μg/ml. and bretylium 20 μg/ml. was non-specific.

Histamine

Concentrations of histamine up to 3 μg/ml. had no effect. Concentrations of histamine greater than 3 μg/ml. produced pronounced relaxation of the bronchial rings after a delay of approximately 30 sec (Fig. 1). In a few preparations the relaxant response was preceded by a very small contraction.

Mepyramine 0·1–1·0 μg/ml. did not inhibit histamine (Table 1 and Fig. 2). Concentrations of propranolol 0·2–5·0 μg/ml., while abolishing isoprenaline relaxation completely, did not antagonize histamine (Fig. 3). The addition of mepyramine and propranolol together did not counteract the histamine relaxation.

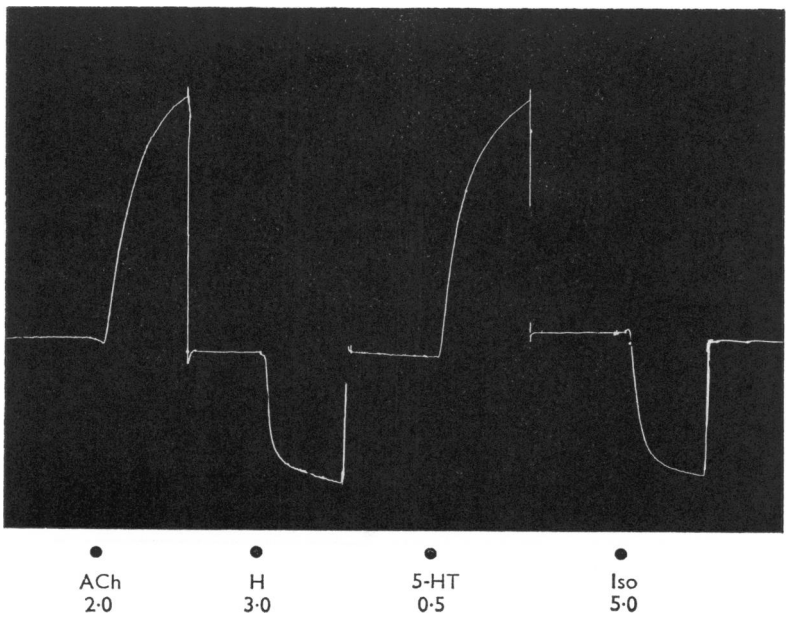

FIG. 1. Isolated bronchial muscle of sheep in 20 ml. Krebs-Henseleit solution at 35° C: 10 min contacts every 30 min. The preparation contracts to acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) and relaxes to histamine (H) and isoprenaline (Iso). Drug doses in μg/ml.

The narcotic barbiturates, thiopentone sodium 20–50 μg/ml. and pentobarbitone 20–50 μg/ml. antagonized the histamine-induced relaxation of bronchial muscle. Acetylcholine (ACh) and 5-HT were partially inhibited but isoprenaline remained unaffected by the barbiturates (Fig. 4). The action of histamine was unaffected by atropine, methysergide, phentolamine, bretylium or cocaine (Table 1).

TABLE 1. *Dose ratios of histamine, 5-HT, acetylcholine and isoprenaline in the presence of antagonists on the isolated bronchial muscle of the sheep*

		Dose ratios of agonists			
Antagonist	Conc. (μg/ml.)	Histamine (relaxation)	5-HT (contraction)	Acetylcholine (contraction)	Isoprenaline (relaxation)
Atropine	0·1	1·0 (4)	1·0 (2)	88 (4)	1·0 (2)
	0·5	1·0 (4)	4·0 (3)	>100 (4)	—
	1·0	1·6 (4)	20·0 (4)	—	1·0 (2)
Mepyramine	0·1	1·0 (4)	1·0 (2)	—	—
	0·5	1·0 (4)	1·0 (2)	1·0 (3)	—
	1·0	1·0 (4)	1·5 (4)	1·5 (3)	1·0 (2)
Methysergide	0·1	1·0 (2)	>100 (4)	1·0 (2)	—
	0·5	1·8 (2)	>100 (4)	2·1 (2)	—
Propranolol	0·2	1·0 (4)	1·0 (4)	1·0 (4)	>100 (4)
	1·0	1·0 (4)	1·0 (3)	1·0 (3)	>100 (4)
	5·0	2·0 (3)	3·0 (3)	1·5 (2)	—
Phentolamine	1·0	1·6 (2)	62·0 (2)	1·0 (2)	—
Bretylium	10·0	1·0 (3)	1·0 (3)	1·0 (3)	—
	20·0	1·5 (3)	1·6 (2)	3·0 (2)	<1·0 (3)
Thiopentone Na	20·0	10·5 (4)	1·5 (4)	2·0 (4)	1·0 (2)
	50·0	20·5 (3)	5·3 (2)	6·5 (2)	1·5 (2)
Pentobarbitone Na	20·0	8·5 (4)	1·0 (4)	2·0 (4)	1·0 (2)
	50·0	10·6 (3)	3·0 (2)	4·0 (2)	2·0 (2)
Cocaine	50·0	1·0 (2)	8·5 (3)	1·5 (2)	1·0 (2)

Figures are means and the number of observations is shown in parentheses. Dose ratio is the ratio of concentrations of agonist which give equal responses in the presence and absence of antagonists.

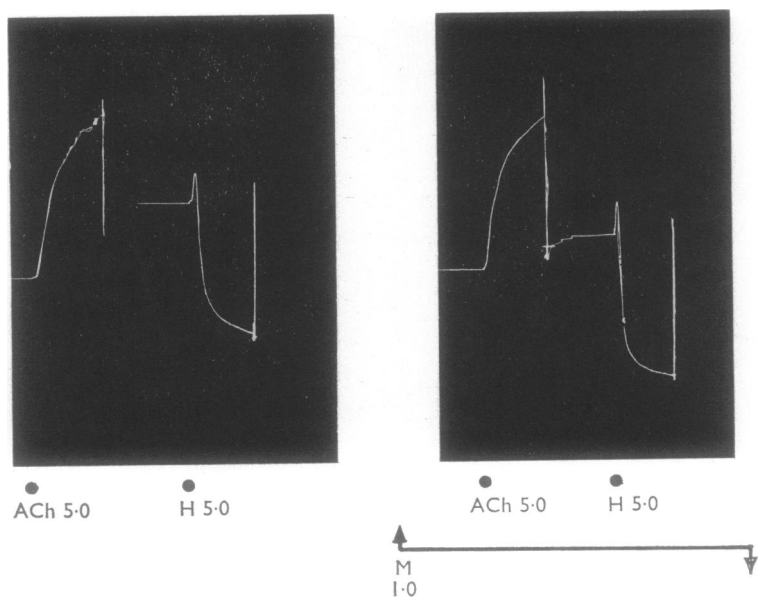

FIG. 2. Isolated bronchial muscle of sheep in 20 ml. Krebs-Henseleit solution at 35° C: 10 min contacts every 30 min. The preparation is contracting to acetylcholine (ACh) and relaxing to histamine (H). Between the arrows mepyramine maleate (M, 1 μg/ml.) was present in the bathing solution. The responses are not affected by the antihistaminic. Drug doses in μg/ml.

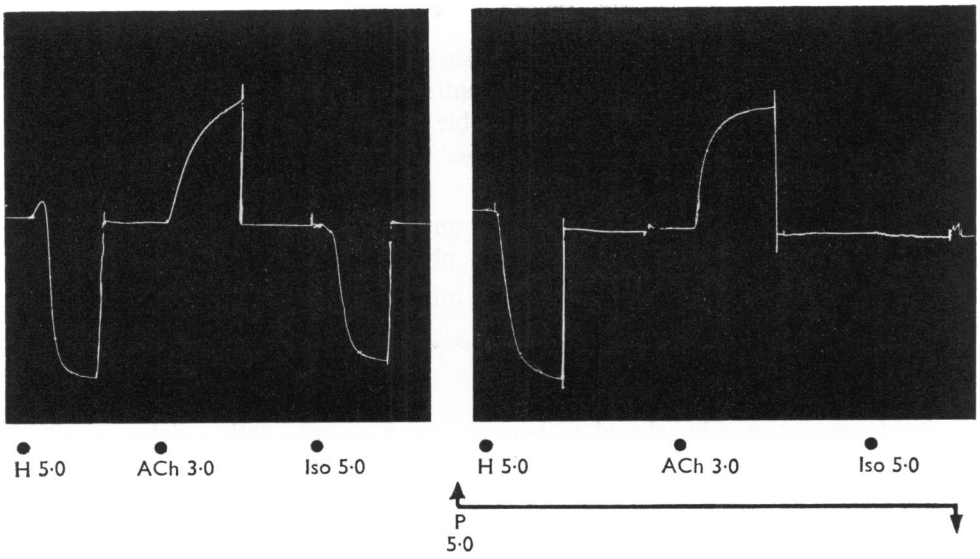

FIG. 3. Isolated bronchial muscle of sheep in 20 ml. Krebs-Henseleit solution at 35° C: 10 min contacts every 30 min. The preparation contracts to acetylcholine (ACh) and relaxes to histamine (H) and to isoprenaline (Iso). Between the arrows propranolol hydrochloride (P, 5 μg/ml.) was present in the Krebs. Propranolol abolished the activity of isoprenaline. The responses to histamine and acetylcholine were unaffected. Drug concentrations in μg/ml.

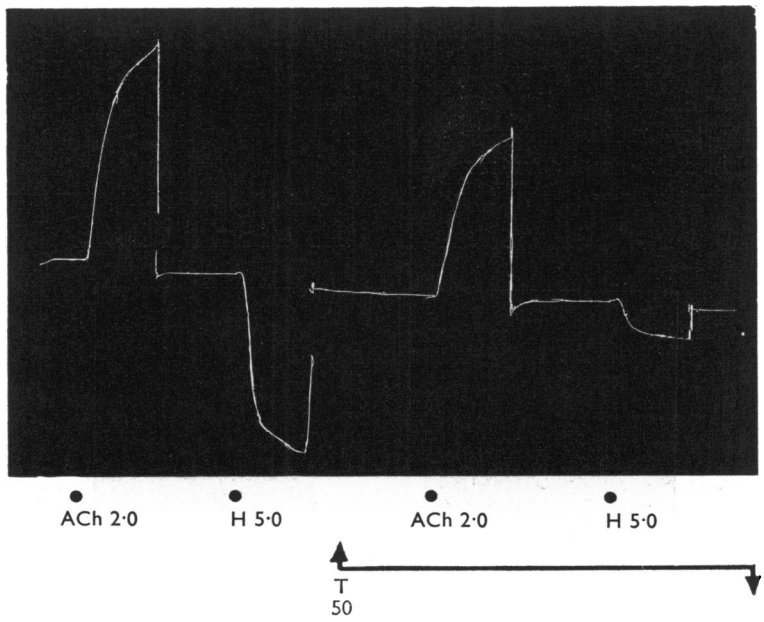

FIG. 4. Isolated bronchial muscle of sheep in 20 ml. Krebs-Henseleit solution at 35° C: 10 min contacts every 30 min. The preparation is contracting to acetylcholine (ACh) and relaxing to histamine (H). Between the arrows thiopentone sodium (T, 50 μg/ml.) was present in solution. Thiopentone inhibited acetylcholine slightly and antagonized histamine to a marked degree. Drug concentrations in μg/ml.

5-Hydroxytryptamine

The bronchial musculature was more sensitive to 5-HT than to any other agonist. 0·5–1·0 μg/ml. of 5-HT caused a strong contraction of the bronchi (Fig. 1) with a delay of approximately 15 sec. Reproducible contractions were difficult to obtain owing to the development of tachyphylaxis. Approximately 2 hr was necessary before restoration of sensitivity.

Methysergide 0·1–0·5 μg/ml. specifically antagonized 5-HT, which was also inhibited by atropine (0·5–1·0 μg/ml.) and by phentolamine 1·0 μg/ml. (Table 1).

The action of 5-HT was also partially inhibited by cocaine, thiopentone and pentobarbitone (each 50 μg/ml.).

Isoprenaline

The bronchial muscle relaxed to isoprenaline 5 μg/ml., with a delay of 10–15 sec (as shown in Fig. 1). Propranolol 0·2–1·0 μg/ml. was a specific antagonist of isoprenaline (Fig. 3). Atropine, mepyramine, methysergide, phentolamine, cocaine, thiopentone and pentobarbitone did not significantly affect the responses of the tissue to isoprenaline. Bretylium 20 μg/ml. potentiated isoprenaline.

Noradrenaline

1–10 μg/ml. was without effect on the tissue.

Bradykinin

1–10 μg/ml. caused no effect, whereas 20 μg/ml. caused a feeble contraction.

Trachea and major bronchi

The sheep trachealis muscle was more sensitive to acetylcholine and less sensitive to 5-HT than the intermediate and lesser bronchi. The minimum concentration of acetylcholine to contract the tracheal muscle was 0·5 to 1·0 μg/ml. The muscle quickly achieved a maximum response to ACh at approximately 2 to 3 μg/ml. and the contraction was of small magnitude. The minimum concentration of 5-HT

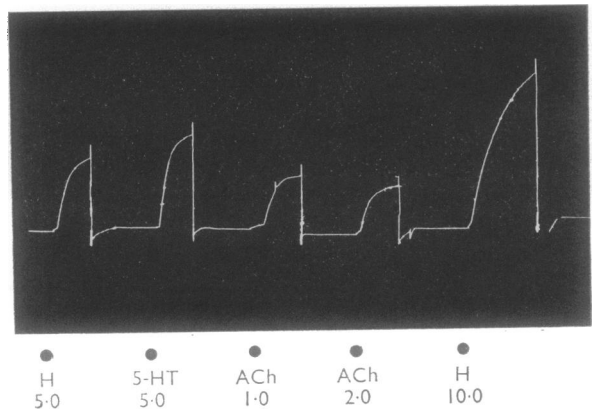

FIG. 5. Isolated trachealis muscle of sheep in 20 ml. Krebs-Henseleit solution at 35° C: 5 min contacts at 30 min intervals. The preparation is contracting to histamine (H), 5-hydroxytryptamine (5-HT) and acetylcholine (ACh). Drug concentrations in μg/ml.

which caused the trachea to contract was 2–5 μg/ml. and this drug showed tachyphylaxis (responses are shown in Fig. 5).

Histamine (2–10 μg/ml.) contracted the tracheal muscle and showed no tendency towards tachyphylaxis. Higher concentrations contracted the muscle maximally and there was no relaxant action of histamine at any concentration up to 30 μg/ml. The action of histamine was specifically inhibited by mepyramine 0·1 μg/ml., giving a dose ratio greater than 100.

Isoprenaline 2 μg/ml. relaxed the trachea and this effect was specifically antagonized by propranolol 0·1 μg/ml.

Thiopentone (50 μg/ml.) alone had no measurable effect on the muscle, but the barbiturate antagonized non-specifically the contractions of the tracheal muscle induced by histamine, 5-HT and acetylcholine.

Discussion

From the data described it appears that the tracheobronchial musculature of sheep responds to histamine in a manner which is different from that of other species. The rodents, dog and man respond with bronchoconstriction, whereas in the cat histamine has been shown to relax the trachea which was contracted by carbachol (Maengwyn-Davies, 1968). However, most of the available data refers to the tracheal muscle rather than bronchi because the small size of the bronchial airways in the " laboratory " species precludes the use of truly bronchial preparations *in vitro*.

The reaction of sheep tissue differs in several ways from that of the cat described by Maengwyn-Davies. In the sheep, histamine caused contraction of the muscles of the trachea and major bronchi and this effect was antagonized specifically by mepyramine. The musculature of the intermediate and terminal bronchi relaxed ; an effect which was not inhibited by the antihistamine. The reactions of feline bronchi *in vitro* do not seem to have been described.

Maengwyn-Davies has postulated, on the results of experiments, using specific antagonists to histamine (mepyramine) and to β-adrenoceptive receptors (pronethalol), that histamine has a dual mode of action on the cat trachea: namely, excitation of its own receptors to induce relaxation and release of catecholamines which excite β-receptors to induce relaxation.

The analysis of the action of histamine on sheep bronchi using antagonists suggests a mechanism which, as far as can be ascertained, has not been described previously in respiratory muscle.

Propranolol 0·2–5·0 μg/ml. did not affect the relaxant action of histamine whereas propranolol 0·1 μg/ml. antagonized completely the relaxant effect of isoprenaline in the same preparation (dose ratio >100). This evidence, coupled with the fact that neither the local anaesthetic concentration of cocaine nor the neuronal blocking concentration of bretylium had any antagonistic action for the histamine relaxation, suggests strongly that histamine is not acting by stimulating sympathetic nerve elements or by releasing catecholamines.

Furthermore, the histamine relaxation was not prevented by concentrations of mepyramine (0·1 μg to 1·0 μg/ml.) normally sufficient to block " typical " histamine receptors (Bartlet & Hassan, 1968 ; Maengwyn-Davies, 1968). Histamine responses were not affected by atropine or methysergide.

It is well known that histamine receptors are divisible into at least two classes. In a study of histamine analogues, Ash & Schild (1966) showed that the molecular structural requirements for histamine combination with receptors in the guinea-pig ileum differed from those for the rat uterus and stomach. Antihistamines fail to antagonize the action of histamine on gastric secretion (Ashford, Heller & Smart, 1949), rat uterus and isolated heart (Dutta, 1949; Trendelenburg, 1960), whereas they block the action of histamine on most smooth muscles, including blood vessels, gastro-intestinal musculature and bronchial muscle of most species. Kier (1968) has postulated that the dual action of histamine is due to isomerism; the two different molecular forms being able to exert distinct biological responses depending on the presence or absence of cell receptors complementary to the particular conformation. This explanation may be analogous to the dual mode of action of the acetylcholine molecule on "muscarinic" and "nicotinic" receptors.

The present evidence suggests strongly that the specific histamine receptors of sheep bronchial muscle differ from those commonly possessed by smooth muscle and may be of a similar type to those in the acid-secreting cells of the stomach. In the absence of any effective antagonist for gastric secretory action of histamine this possibility could not be confirmed or denied.

The report of Alexander *et al.* (1967) that histamine caused increased resistance to pulmonary inflation in anaesthetized sheep appears to conflict with the present observations of bronchial relaxation *in vitro*. In sheep anaesthetized with cyclopropane in oxygen the histamine-induced pulmonary resistance was usually quantitatively less than in those sheep which had been anaesthetized solely with the barbiturates thiopentone and/or pentobarbitone (unpublished observations). It was therefore of considerable interest and importance to find that both thiopentone and pentobarbitone (20 μg/ml.) inhibited the ability of histamine to relax sheep bronchial muscle *in vitro*. Greater concentrations of the barbiturates (50 μg/ml.) inhibited also the contractile responses of acetylcholine and 5-hydroxytryptamine on the bronchi; weakly antagonized histamine, acetylcholine and 5-HT on the trachealis muscle, but did not modify the responses due to isoprenaline.

Fletcher, Flacke & Alper (1968) have demonstrated that thiopentone, ether and halothane all relax the isolated tracheal muscle of the guinea-pig and inhibit the contractile responses of acetylcholine and histamine in this preparation. Adriani & Rovenstine (1943) reported that thiopentone contracted the bronchi of rat, dog and man. Thus it would appear that the influence of anaesthetic agents on the contractile response of airway muscle varies between species.

It is clear that the resistance to pulmonary inflation in any intact animal is the algebraic sum of changes in tone throughout the arbitrary "compartments" of the respiratory tract from the larynx to the alveolar surfaces. The final measurement of airway resistance will depend on the extent of the participation of each "compartment." In the sheep the trachea and major bronchi tend to increase resistance and the lesser bronchi and bronchioles decrease resistance in the presence of histamine. The action of barbiturate in preferentially antagonizing the histamine-induced bronchial-bronchiolar relaxation will predispose to tracheobronchial contraction and increase in inflationary resistance; which is what Alexander *et al.* (1967) observed.

5-Hydroxytryptamine consistently caused contraction of muscles in all parts of the sheep's tracheobronchial tree. The smaller bronchi and bronchioles were more

sensitive to 5-HT than to any other agent tested, whereas the tracheal muscle was particularly insensitive to 5-HT. Methysergide (0·1 μg/ml.) was a specific antagonism for 5-HT (dose ratio >100) and atropine (1·0 μg/ml.) had selective antagonism for 5-HT also (dose ratio $=20{\cdot}0$). It is possible therefore that 5-HT may be acting on sheep airway muscle in two ways: (i) by combining with specific 5-HT receptors and (ii) indirectly through elements of the parasympathetic nervous system, thus exciting muscarinic receptor mechanisms.

The action of acetylcholine was to contract the tracheobronchial muscle and isoprenaline caused relaxation, effects which were specifically blocked by atropine and propranolol respectively.

The sheep tracheobronchial preparation was extremely insensitive to bradykinin, 20 μg/ml. being necessary to produce a feeble contraction, which appeared to be maximal.

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