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## RESPONSES OF ISOLATED BRONCHIAL MUSCLE TO GANGLIONICALLY ACTIVE DRUGS

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It is generally accepted that the efferent innervation of the bronchial musculature is by branches from the vagus nerve and from the thoracic sympathetic trunk, entering the lung at its hilum (Macklin, 1929). The trachea is thought to have a similar supply. The existence of ganglion cells in the lung, distributed along the branches of the bronchial tree, has been known since the time of Remak (1844). The autonomic innervation appears to follow the usual pattern, in that the vagus fibres entering the lung are preganglionic and form synapses with Remak's ganglia within the lung; processes from these cells end on the smooth muscle cells in the walls of the bronchi. The sympathetic supply consists of post-ganglionic fibres which end directly on the smooth muscle of the bronchi.

Although it is usually held that the vagus nerve mediates bronchoconstriction and the sympathetic supply bronchodilatation, there is evidence that this simple picture is not completely correct. For instance, some workers have observed that under certain conditions excitation of the vagus, instead of producing the usual bronchoconstriction, may elicit bronchodilatation. There is little doubt that some of the earlier workers in fact stimulated not the vagus alone, but the vagosympathetic trunk. Further differences of opinion may have arisen from failure to realize the difficulty of demonstrating bronchodilatation in a preparation which has little tone under the experimental conditions. Dixon & Brodie (1903), who were aware of both these difficulties, concluded that the vagus nerve itself contained both bronchodilator and bronchoconstrictor fibres. This conclusion has subsequently been confirmed in guinea-pigs, cats, dogs and rabbits, the bronchodilator action of the vagus being more readily demonstrable after a dose of some bronchoconstrictor drug. Since the vagal bronchomotor fibres have been shown to end on the

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ganglion cells of the lung (Larsell & Mason, 1921) the question arises whether these ganglion cells play a part in mediating both types of vagal action, or whether their function is solely bronchoconstrictor.

The present work was designed to study the characteristics of the ganglionic tissue within the trachea and large bronchi, using isolated preparations of trachea and bronchi from guinea-pig and cat. Pharmacological evidence will be presented that at least two types of ganglion cell, adrenergic and cholinergic, are present in these tissues.

#### METHODS

*Material.* Guinea-pig tracheae and bronchi were obtained from animals freshly stunned and bled out. Cat trachea was taken from animals which had been anaesthetized with ether for other purposes; large bronchi from these animals were taken at the end of experiments under ether or chloralose anaesthesia, lasting for 2-3 hr. We are grateful to Professor R. S. Pilcher for two human lungs, each removed at pneumonectomy for carcinoma in one lobe.

*Isolated preparations.* Chains of rings cut from bronchi and trachea, of the type described by Epstein (1932) and Castillo & de Beer (1947) were employed. Bronchi from guinea-pigs, cats, and humans were dissected free from lung tissue, and tied in chains with loops of cotton. Rings cut from guinea-pig trachea were orientated alternately and tied tightly with cotton so that the muscle strips were aligned. With cat trachea and the larger human bronchi single rings were often adequate. Rings cut by sectioning the trachea at intervals of 8-10 mm, containing two or three cartilaginous rings, were sometimes used; these were suspended by means of stitches put into the walls with a small curved needle.

The preparations were suspended in isolated organ baths, and the tonus level recorded with light balsa-wood frontal-writing levers recording on a lightly smoked drum, rotating at 1 mm/min. The solution used was Krebs-Henseleit solution: composition (g/l.), NaCl 6.87, KCl 0.42, CaCl<sub>2</sub> 0.28, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.29, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O 0.18, NaHCO<sub>3</sub> 2.10, glucose 1.00, aerated with oxygen and 5% CO<sub>2</sub> at 37° C. The bath volume was 50 ml.

The preparations were usually set up within 1 hr of removal from the animal, and then left for a further hour before testing. With guinea-pig trachea chains a marked rise in tone occurred during this time. The preparations responded to drugs for 10-12 hr. A special difficulty sometimes arose from the relatively high tone of the preparation of guinea-pig tracheal muscle. Not infrequently, washing out the bath led to a considerable change of tone, commonly a reduction, followed by a return to normal over a period of 10-20 min. In the absence of washing out only slow drifts of tone occurred, and there was no difficulty in distinguishing them from bronchoconstrictor or bronchodilator responses.

The Ringer's solution used by Epstein (1932) proved to be unsatisfactory. Preparations of guinea-pig trachea immersed in it were insensitive and soon ceased to respond to drugs at all.

*Drugs.* Drug concentrations were expressed in terms of the whole salt unless the contrary is stated. For the pA<sub>2</sub> determinations molar concentrations were computed in terms of C<sub>33</sub>H<sub>37</sub>O<sub>5</sub>N<sub>5</sub>, CH<sub>3</sub>SO<sub>3</sub>H for dihydroergotamine methanesulphonate and of C<sub>33</sub>H<sub>35</sub>O<sub>5</sub>N<sub>5</sub>·½(C<sub>4</sub>H<sub>6</sub>O<sub>4</sub>) for ergotamine tartrate.

The drugs used were: acetylcholine iodide, anagyrine hydriodide, ascorbic acid, atropine sulphate, barium chloride, B<sub>1</sub>-pyrimidine hydrochloride (2-methyl-4-amino-5-methylamine pyrimidine; Roche Products), choline chloride, coniine hydrochloride, cytisine perchlorate, dibenamine hydrochloride, dibenzylamine hydrochloride (Smith, Kline and French Laboratories, Ltd.), dihydroergotamine methanesulphonate, egg albumin (flake), ergometrine maleate, ergotamine tartrate, ergotoxine ethanesulphonate, hexamethonium bromide, histamine dihydrogen phosphate, L-adrenaline base, L-noradrenaline bitartrate monohydrate (Bayer Products, Ltd.), lobeline hydrochloride (Sandoz Products), mepyramine acid maleate (May and Baker, Ltd.),

nicotine acid tartrate, phentolamine hydrochloride (7337, Rogitine; Ciba Laboratories), pilocarpine nitrate, physostigmine sulphate, prosympal (883F, diethylamino-methylbenzodioxane hydrochloride; May and Baker, Ltd.), sparteine sulphate, tetramethylammonium bromide, tolazoline hydrochloride (Priscol; Ciba Laboratories).

Nicotine acid tartrate was made up 1% or 10% (w/v), in the Krebs-Henseleit solution. Equivalent doses of tartaric acid were totally inactive under the experimental conditions. Adrenaline was dissolved in equivalent dilute HCl, and dilutions prepared in distilled water containing ascorbic acid  $10^{-5}$ ; noradrenaline was made up in ascorbic acid  $10^{-5}$ . Fresh solutions of these drugs were prepared every 4-6 hr. Lobeline hydrochloride was dissolved at 1% in distilled water with the aid of a drop of dilute HCl. Dibenzylamine was made up in 0.01 N-HCl. Ergotamine, dihydroergotamine, ergometrine, phentolamine and dibenamine were diluted from ampoules. Solutions of the remaining drugs were prepared in distilled water. The ergotoxine ethanesulphonate used was a routine production batch (Burroughs Wellcome), satisfied the B.P. requirements, and was estimated polarimetrically to contain 85% ergocornine, the remainder probably representing ergocristine.

We are grateful to the drug firms mentioned in the above list for supplies of the pure drugs.

## RESULTS

### *Bronchodilator response of guinea-pig trachea, to nicotine*

The principal action of nicotine on the guinea-pig tracheal chain is to produce a transient relaxation of the muscle. This response is obtained with concentrations of nicotine between 20 and 200  $\mu\text{g/ml}$ . and is illustrated in Fig. 1.

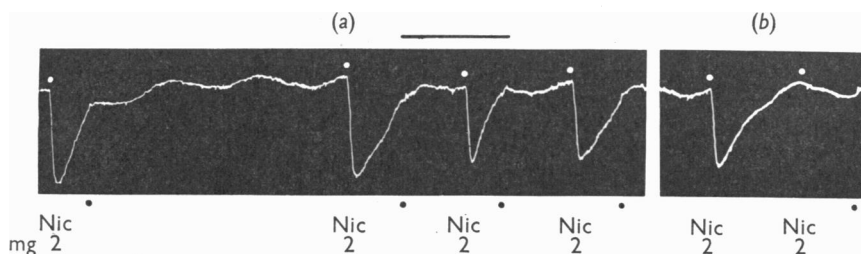


Fig. 1. Guinea-pig tracheal chain. In all figures, white dot marks the time of injection; ● denotes beginning of washing out; the black line at top of tracing gives 30 min time scale; bath capacity is 50 ml. (a) Successive responses to nicotine 2 mg added to bath; (b) 50 min later, abolition of nicotine response by leaving a previous dose in bath.

The response consists of an initial rapid relaxation followed by a slow return to the resting base line. If the nicotine is kept in the bath, the tracheal muscle is unresponsive to a further similar dose of nicotine (Fig. 1b). If the bath is washed out repeatedly, the relaxation response to nicotine can be repeated after an interval of 30-45 min. Thus with regular injections every 40 min, leaving the nicotine in contact for about 5 min, repeated constant responses to constant doses of nicotine or graded responses to graded doses may be obtained.

The manner in which the tissue responded rapidly to nicotine, then recovered from it, and finally became for a fairly prolonged period resistant to further doses of nicotine, strongly recalled the responses of ganglion cells to this drug.

We therefore proceeded to test further the possibility that there might be situated in the tissue ganglion cells possessing a bronchodilator action.

*Hexamethonium.* The response to nicotine was reduced by hexamethonium when present in a concentration of  $0.1 \mu\text{g/ml}$ . (Fig. 2*a*). Such low concentra-

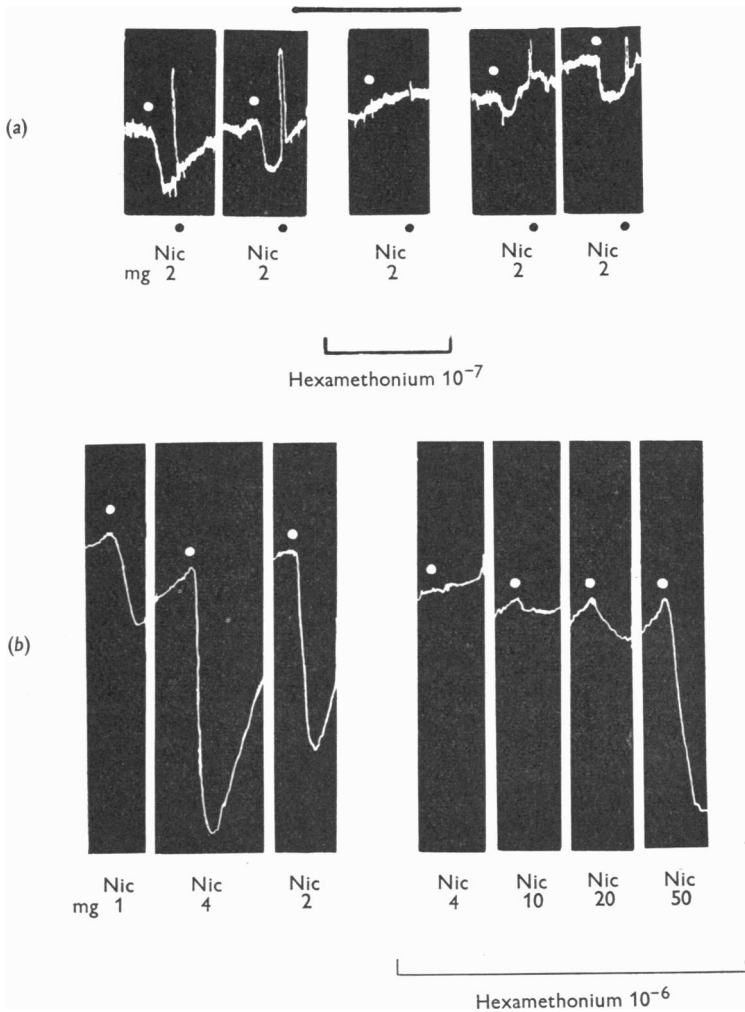


Fig. 2. Guinea-pig tracheal chain. (a) Effect of hexamethonium  $10^{-7}$  on responses to 2 mg nicotine at 45 min intervals, hexamethonium added to bath 30 min before third dose of nicotine; (b) effect of hexamethonium  $10^{-6}$  on various doses of nicotine, showing approximately 20-fold antagonism.

tions of hexamethonium rarely show any action other than that of ganglion paralysis. The antagonism of the nicotine relaxation by these concentrations of hexamethonium therefore increased the likelihood that the nicotine action is

produced by excitation of bronchodilator ganglion cells situated in close relation to the tracheal muscle.

The antagonism between hexamethonium and nicotine appeared to be competitive, progressively higher concentrations of hexamethonium blocking responses to progressively larger doses of nicotine. For example, in one experiment hexamethonium  $0.1 \mu\text{g/ml}$ . abolished responses to nicotine  $50 \mu\text{g/ml}$ . but merely reduced the responses to nicotine  $100$  and  $200 \mu\text{g/ml}$ . On the other hand, in the experiment shown in Fig. 2*b* a large dose of hexamethonium,  $1.0 \mu\text{g/ml}$ ., abolished responses to nicotine  $40$ ,  $80$  and  $200 \mu\text{g/ml}$ , whereas nicotine  $400$  and  $1000 \mu\text{g/ml}$ . still had an effect.

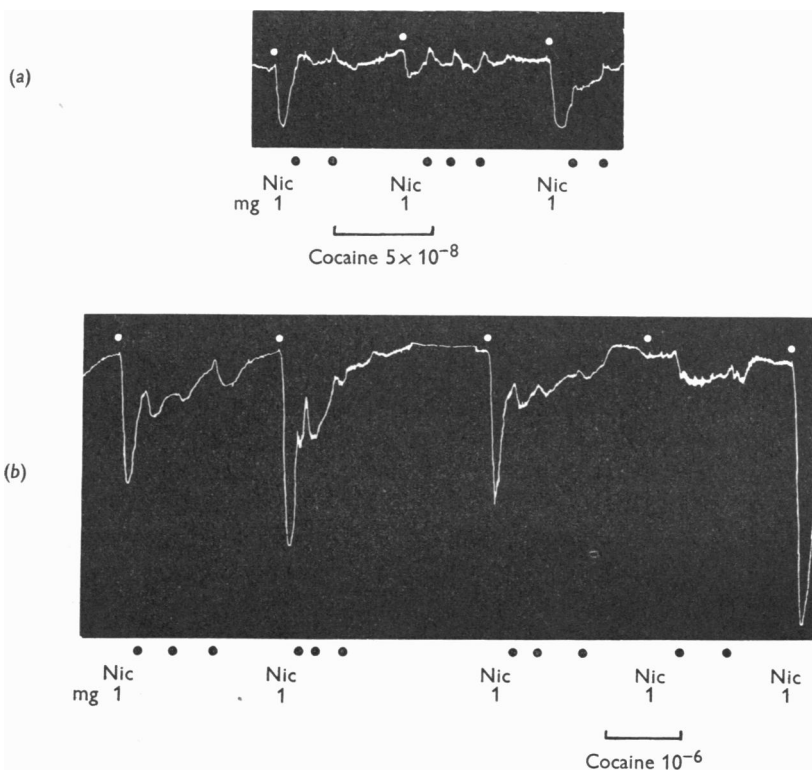


Fig. 3. Guinea-pig tracheal chain. (a) Effect of cocaine  $5 \times 10^{-8}$  on response to nicotine; (b) effect of cocaine  $10^{-6}$  on response to nicotine.

*Cocaine.* If the response to nicotine is mediated by nervous tissue, then it should be reduced or abolished by local anaesthetic drugs. Figure 3 shows the effect of cocaine on the response; the nicotine response was reduced by a low concentration of cocaine ( $0.05 \mu\text{g/ml}$ .) and abolished by cocaine in a concentration of  $1.0 \mu\text{g/ml}$ .

*Nicotinic alkaloids.* We have already mentioned that nicotine tends to block its own action on the tracheal muscle (Fig. 1*b*). It is known that related alkaloids can antagonize other responses to nicotine (Dale & Laidlaw, 1911-12) and the action of a number of these alkaloids was examined. It was found that lobeline 1  $\mu\text{g}/\text{ml}$ ., coniine 0.5  $\mu\text{g}/\text{ml}$ ., cytisine 5  $\mu\text{g}/\text{ml}$ ., sparteine 0.5  $\mu\text{g}/\text{ml}$ . and anagryrine 2  $\mu\text{g}/\text{ml}$ . all abolished the nicotine response. These alkaloids did not affect bronchodilator responses to adrenaline or noradrenaline.

*Sympatholytic drugs.* Since the response to nicotine was bronchodilator, the ganglion cells concerned might be supposed to mediate the response by liberating sympathin at their post-ganglionic endings. Some difficulty was expected in testing this conclusion with sympatholytic drugs, for two reasons. It is commonly held that such drugs are only effective against the stimulant actions of adrenaline; and the pharmacological effect of many of these drugs is a complex mixture of sympatholytic, sympathomimetic, and potentiating actions. It was therefore judged necessary first to examine a number of sympatholytic drugs in order to select drugs which could be used to antagonize, if possible differentially, the relaxant effects of adrenaline and noradrenaline on the tracheal chain.

The results of this survey are shown in Table 1. It was found that ergotoxine would antagonize the bronchodilator action of adrenaline, but not that of noradrenaline. This drug is unsatisfactory to use because of its low solubility; it tends to be precipitated after addition to the bath. It was therefore added to the bath as a fine aqueous suspension to give a final 'concentration' of 100  $\mu\text{g}/\text{ml}$ .; under these conditions the drug was precipitated, but presumably gave a saturated solution. Ergotoxine was unsuitable for quantitative work, but ergotamine and dihydroergotamine both antagonized adrenaline over fairly wide ranges of concentration. The results with these alkaloids against noradrenaline were rather irregular. In a single test, ergometrine was inactive against both amines. Prosympal (883 F) and phentolamine were both inactive against the amines in the concentrations tested. Dibenamine and dibenzyline showed mixed potentiating and antagonistic actions, and became precipitated in the bath. Mepyramine was in general inactive against adrenaline but potentiated noradrenaline in two experiments. Tolazoline potentiated adrenaline at most of the levels of dose tested.

Ergotoxine used as just described, to give a concentration in the bath of something less than 100  $\mu\text{g}/\text{ml}$ ., abolished the bronchodilator response of the guinea-pig trachea to nicotine, and also the dilator response to adrenaline, leaving the response to noradrenaline unaffected (Fig. 4*a*). This indicated that the nerve endings mediating the nicotine response were adrenergic, and suggested that the transmitter was adrenaline itself.

In a number of experiments the actions of low concentrations of dihydroergotamine and ergotamine on responses to adrenaline and nicotine were

TABLE 1. Antagonism of bronchodilator actions of adrenaline and noradrenaline by sympatholytic drugs. Summary of experiments on guinea-pig trachea

Antagonist	Concentration	Action against		
		Adren- aline	Noradren- aline	
Dibenamine	$10^{-5}$	0	0	Potentiated adrenaline and norad.
	$10^{-4}$	0	.	Precipitated; potentiated
	$10^{-3}$	+	.	Precipitated
Dibenzyline	$10^{-6}$	0	0	Potentiated adrenaline and norad.
	$10^{-5}$	0	0	Potentiated adrenaline
	$4 \times 10^{-5}$	+	.	Precipitated
Dihydroergotamine	$10^{-6}$	0	0	.
	$10^{-7}$	0	+	.
	$2 \times 10^{-7}$	+	0	.
	$3 \times 10^{-7} - 8 \times 10^{-7}$	+	.	.
	$10^{-6}$	+	+	.
	$2 \times 10^{-6}$	+	.	.
	$5 \times 10^{-6}$	+	0	.
	$10^{-5}$	.	0, +	.
Ergotamine	$1.6 \times 10^{-5}$	.	.	.
	$2 \times 10^{-7}$	0	.	.
	$5 \times 10^{-7}$	0, +	.	.
	$8 \times 10^{-7}$	+	.	.
	$10^{-6}$	+	0	.
	$4 \times 10^{-6}$	.	+	.
	$5 \times 10^{-6}$	+	.	.
	$8 \times 10^{-6}$	.	0, +	.
	$10^{-5}$	+	0	Potentiated noradrenaline
	$1.6 \times 10^{-5}$	.	0	.
Ergometrine	$2 \times 10^{-5}$	.	+, +	.
	$10^{-6}$	0	0	.
Ergotoxine	$10^{-5}$	0	0	.
	$10^{-4}$	++	0	Precipitated
Mepyramine	$1.6 \times 10^{-8}$	.	0	.
	$8 \times 10^{-8}$	0	.	.
	$1.6 \times 10^{-7}$	.	0	.
	$8 \times 10^{-7}$	0, +	0	Potentiated noradrenaline
	$8 \times 10^{-6}$	0	0	.
Phentolamine	$2 \times 10^{-6}$	0	0	.
Prosypal	$10^{-6} - 10^{-5}$	0	0	.
	$10^{-4}$	0	.	.
Tolazoline	$10^{-6} - 2 \times 10^{-6}$	0	.	.
	$5 \times 10^{-6} - 2 \times 10^{-5}$	0	.	Potentiated
	$5 \times 10^{-5}$	0	.	.
	$10^{-4}$	0	0	Potentiated adrenaline and norad.

+ = Antagonism; 0 = no antagonism.

compared. One such experiment is illustrated in Fig. 4b, where it will be seen that dihydroergotamine  $0.2 \mu\text{g/ml.}$  barely affected the responses to adrenaline and nicotine, while in higher concentrations,  $1.0 \mu\text{g/ml.}$ , the antagonist reduced both responses to approximately the same extent. In general the effects of these ergot alkaloids in antagonizing the nicotine response paralleled their effects in antagonizing equivalent responses to adrenaline; considerably

higher concentrations of antagonist were required to affect the response to noradrenaline materially.

The activities of the two most suitable antagonists, ergotamine and dihydroergotamine, against adrenaline, noradrenaline, and nicotine, were assessed quantitatively. In addition, since Lockett (1957) has produced evidence in the

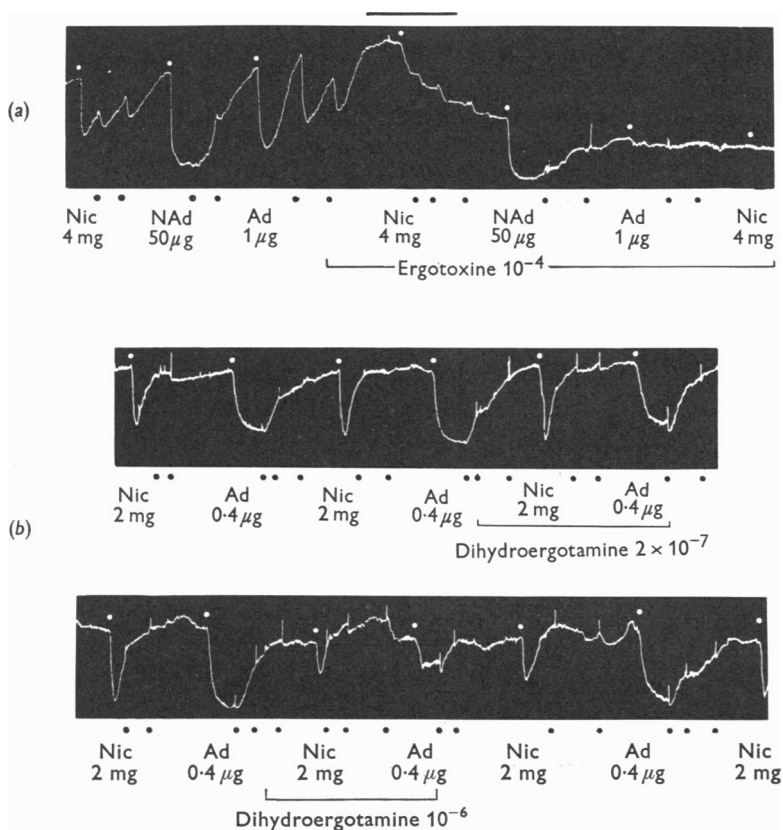


Fig. 4. Guinea-pig tracheal chain. (a) Action of ergotoxine  $10^{-4}$ , abolishing response to nicotine and adrenaline but not that to noradrenaline; (b) action of dihydroergotamine  $2 \times 10^{-7}$  and  $10^{-6}$  on responses to adrenaline and nicotine.

intact cat that isoprenaline mediates the responses of the bronchi to sympathetic stimulation, we also examined the antagonism of these sympatholytics to isoprenaline. The results are given in the  $pA_2$  notation of Schild (1947*a, b*) which expresses the activity of an antagonist as the negative logarithm of the concentration required to reduce the response of a double dose of the active drug to the same level as the response of a single dose of active drug in the absence of antagonist. The ergot compounds caused a rise in base line, which prevented these measurements being fully accurate when the antagonist was



added. The figures for  $pA_2$  given represent estimates obtained as in Fig. 5; this shows the effects of a concentration of antagonist definitely greater than the  $pA_2$ , of a concentration approximately equal to the  $pA_2$ , and of a concentration definitely less than the  $pA_2$ . In records where the base line was elevated by the ergot alkaloid, response heights were estimated from the base line immediately before addition of the dose of nicotine. Two series of determinations were performed; in the first the antagonist was allowed to remain in contact with the tissue for 20 min before the dose of active drug was given, whereas

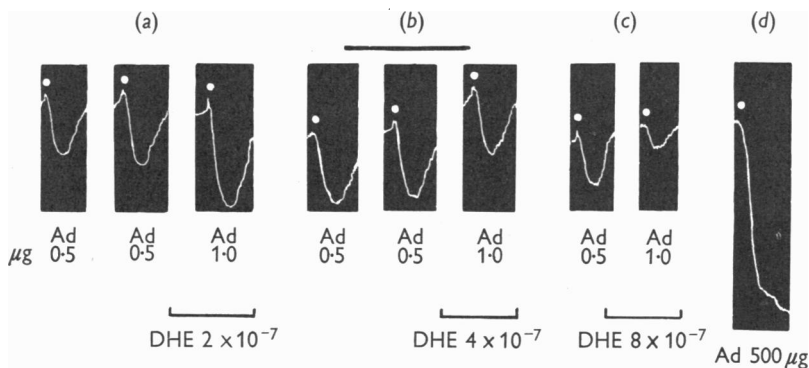


Fig. 5. Guinea-pig tracheal chain. Part of an estimation of  $pA_2$  for dihydroergotamine against adrenaline. The last panel gives the effect of a large dose of adrenaline, showing that sub-maximal responses were used.

in the second series a 30 min contact was used. The results in the two series did not differ materially and have been combined in Fig. 6 (showing the individual observations), and in Table 2, which summarizes the  $pA_2$  values deduced. With the antagonisms between the ergot compounds and noradrenaline the results were rather irregular, and potentiation sometimes occurred with the highest concentrations of antagonist. The values for these antagonisms in Table 2 are therefore shown in parentheses. The principal result to be deduced from Fig. 6, and Table 2, is that the absolute activities of the two antagonists against nicotine and against adrenaline or isoprenaline were of the same order, but they differed widely from the absolute activities against noradrenaline.

These quantitative experiments therefore confirm the results obtained with ergotamine: (a) that the response to nicotine is mediated by a sympathomimetic amine; and (b) that the amine concerned resembles adrenaline rather than noradrenaline. Adrenaline, however, is not the only candidate; isoprenaline also has the pharmacological properties required.

*Sympathetic potentiators.* The association between local anaesthetic activity, anti-amine oxidase activity, and potentiation of sympathomimetic activity is well known. It was therefore expected that it would be difficult to demonstrate

potentiation of the response to nicotine by known adrenaline potentiators. This proved to be the case. For example, with cocaine the lowest concentration with which we were able reproducibly to potentiate adrenaline responses on the trachea was about 100  $\mu\text{g}/\text{ml}$ ., whereas this local anaesthetic will often antagonize the nicotine response in concentrations as low as 0.02  $\mu\text{g}/\text{ml}$ .

Rosa & McDowall (1951) reported that ascorbic acid increases the response of isolated human bronchial muscle to adrenaline. We were unable to confirm

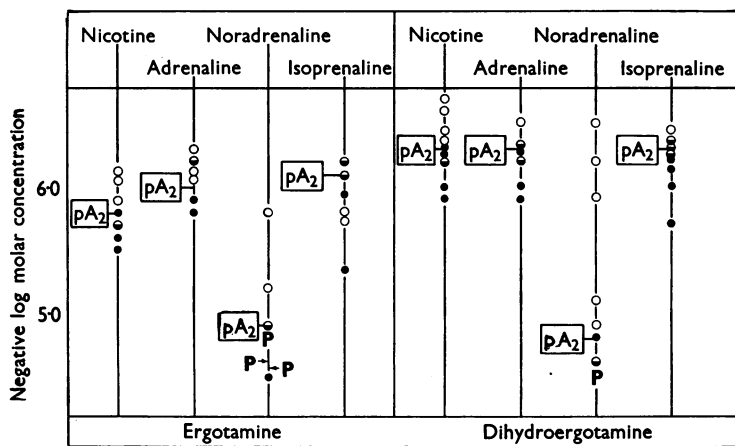


Fig. 6. Diagram of results of experiments to estimate  $pA_2$  of ergotamine and dihydroergotamine against nicotine, adrenaline, noradrenaline, and isoprenaline.  $\circ$ ,  $>pA_2$ ;  $\ominus$ ,  $=pA_2$  approx.;  $\bullet$ ,  $<pA_2$ ; P, potentiation.

TABLE 2.  $pA_2$  values and relative potencies of sympatholytics in the antagonism of bronchodilator effects on the guinea-pig trachea

		Nicotine	Adrenaline	Noradrenaline	Isoprenaline
$pA_2$	Ergotamine	5.8	6.0	(4.9)	6.1
	Dihydroergotamine	6.3	6.3	(4.8)	6.3
Relative potency	Ergotamine	60	100	(8)	130
	Dihydroergotamine	200	200	(6)	200

Ergotamine versus adrenaline = 100.

this on guinea-pig trachea, and the action of ascorbic acid on the nicotine response was difficult to assess, because irregular changes in the base line followed the addition of ascorbic acid to the bath.

*Other drugs.* *Atropine* in concentrations from 1 to 100  $\mu\text{g}/\text{ml}$ . reduced the bronchodilator nicotine response without affecting the response to adrenaline or noradrenaline. The minimum concentration of atropine found to be active in this respect was more than one hundred times that required to reduce an acetylcholine contraction of the trachea. *Mepyramine* had a rather irregular influence on the response to adrenaline and noradrenaline, sometimes potentiating the action of the latter. It reduced the nicotine response. *Tolazoline*,

which regularly potentiated the action of adrenaline, had an anti-nicotinic action in concentrations of 10 and 20  $\mu\text{g/ml}$ . *Prosympal*, which was inactive in concentrations up to 100  $\mu\text{g/ml}$ . against the bronchodilator responses to noradrenaline and adrenaline, antagonized nicotine bronchodilatation at 1 and 2  $\mu\text{g/ml}$ . *Phentolamine* was similarly inactive against the sympathetic amines at 2  $\mu\text{g/ml}$ ., but reduced the nicotine response.

The general pattern of action of this group of drugs, depression of the response to nicotine but not to sympathetic amines, raises one of the recurrent difficulties in work of this kind with imperfectly specific drugs. Many such drugs are able to produce local anaesthesia, a property common to a wide range of aromatic amines (Burn, 1956). Such an action, either by depressing conduction in post-ganglionic nerve fibres or by block at the ganglion synaptic area (Harvey, 1939) would lead to a reduction of the action of nicotine in our experiments, without necessarily directly affecting the response of the bronchial smooth muscle itself to drugs such as adrenaline. It is known that atropine, mepyramine and prosympal have a local anaesthetic action, the first two being comparable in potency with procaine (Bacq, 1935; Wierzuchowski & Bielinki, 1939; Dews & Graham, 1946; Dutta, 1949). Tolazoline and phentolamine possess a wide variety of actions (Goodman & Gilman, 1955), and although their local anaesthetic potency does not appear to have been studied, it would be surprising, from the general properties of such drugs, if they did not possess it in some degree. Since cocaine in a dilution of one in twenty million reduced the nicotine response, a potency one-twentieth of that of cocaine, or less, would suffice to account for the actions of these compounds. We have, therefore, attributed the results described to their local anaesthetic action on the nervous tissue in the bronchial muscle preparations.

Thus the bronchodilator response of the guinea-pig tracheal chain to nicotine possesses the pharmacological characteristics of an excitation of ganglion cells situated in the tissue, releasing adrenaline at their post-ganglionic endings. These characteristics may be summarized as follows:

- (1) The tissue recovers from the effect of nicotine without removal of the drug from the bath and in this state is refractory to the addition of further doses of nicotine.
- (2) The effect of nicotine is blocked by very low doses of hexamethonium and by 'nicotinic' alkaloids.
- (3) The response is unaffected by specific concentrations of atropine.
- (4) The response is reduced by ergotoxine, ergotamine and dihydroergotamine; the effects of these sympatholytic drugs parallel their effects on adrenaline and isoprenaline responses, but not their effects on noradrenaline responses.
- (5) The response is abolished by drugs having a local anaesthetic action.

*Bronchoconstrictor response of guinea-pig trachea to nicotine*

It was expected at the outset that cholinergic ganglion cells on the trachea would give rise to a bronchoconstrictor response to nicotine. The most prominent response was the one we have just described, bronchodilatation. It was, however, noted that with fresh preparations the earliest responses were sometimes diphasic in character, relaxation of the smooth muscle being preceded by a brief and small contraction. Examples of this diphasic response are shown in Fig. 7 *a, b*. A similar type of response was occasionally seen after the tone of the preparation had been temporarily reduced by some means. Fig. 7 *c* shows a diphasic response to nicotine obtained during a reduction of tone persisting after a response to adrenaline. Fig. 7 *d* shows a similar diphasic response produced by nicotine during a depression of tone after a response to acetylcholine.

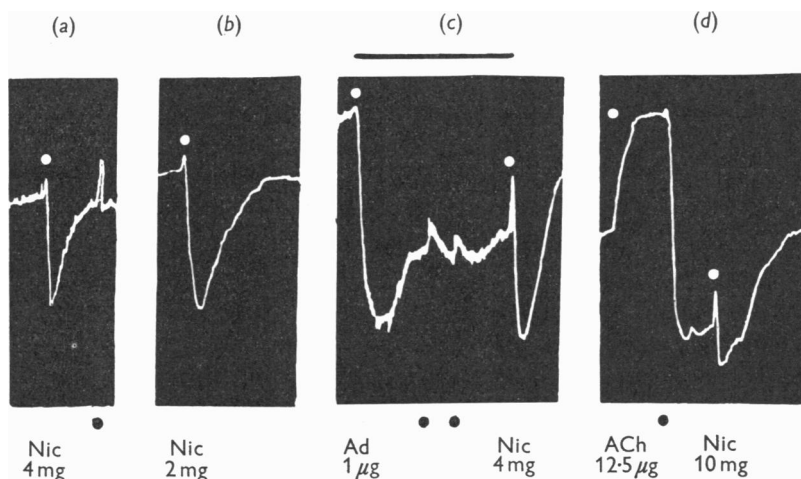


Fig. 7. Guinea-pig tracheal chain. Diphasic responses to nicotine obtained (*a*) and (*b*) spontaneously in two preparations; (*c*) and (*d*) during a persisting relaxation following adrenaline or acetylcholine washing out respectively.

This suggested that a number of cholinergic ganglion cells were responding to nicotine in our experiments, but that the response was greatly overshadowed by that of the adrenergic ganglion cells. We therefore examined the action of eserine, to see whether this drug would influence the response of the tracheal chain to nicotine. This showed at once the presence of a cholinergic response, the bronchoconstrictor component being greatly enhanced, so far as to cause an actual reversal of the response to nicotine (Fig. 8). This bronchoconstrictor response to nicotine resembled in its general pharmacological properties the bronchodilator response, in that it was antagonized by the presence of a

previous dose of nicotine in the bath, by specific concentrations of hexamethonium and by specific concentrations of the appropriate antagonist of the transmitter, in this case atropine.

*Other responses to nicotine and nicotinic drugs.* An additional response by the tracheal chain to low doses of nicotine was observed in a number of experiments. When a preparation was tested within an hour or two of its being set up, using doses of nicotine between 1 and 10  $\mu\text{g}/\text{ml}$ ., it responded

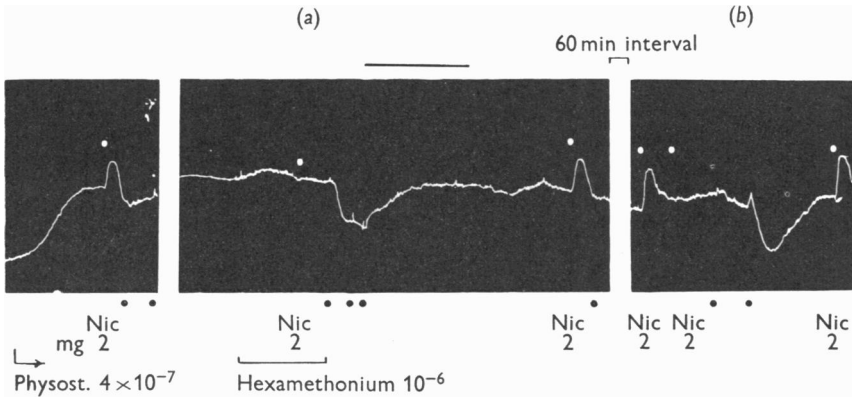


Fig. 8. Guinea-pig tracheal chain. Tone raised by physostigmine  $4 \times 10^{-7}$ . Effect of nicotine (a) before, during and after hexamethonium  $10^{-6}$ ; (b) in the presence of a previous dose of nicotine.

with a very small slow contraction, of the order of 5% of the maximal contraction to barium. This contraction could not be repeated more than once or, rarely, twice, and it was resistant both to hexamethonium in concentrations up to 400  $\mu\text{g}/\text{ml}$ . and to atropine up to 10  $\mu\text{g}/\text{ml}$ . This small contraction seemed to be somewhat more easily obtained when the tracheal chains were so cut that the individual rings were wider than usual and each included three or four cartilaginous rings. Since these responses are not antagonized by hexamethonium or atropine, it is considered that they are not typical ganglion-cell responses, nor muscarinic actions.

Other alkaloids related to nicotine also exhibited some anomalous effects. The principal actions of these alkaloids on smooth muscle are generally thought to be mediated by initial stimulation followed by secondary depression of autonomic ganglia. In our experiments lobeline, cytisine, coniine, anagryrine, and sparteine were found to give contractions of tracheal chains in moderate dose, and lobeline and anagryrine gave relaxations with higher doses. With all these drugs except lobeline the responses were relatively small. They were unlike the rapid transient nicotine responses and were resistant to hexamethonium. Further, the bronchoconstrictor responses were not antagonized

by atropine, and the bronchodilator responses resisted dihydroergotamine. It was concluded that although these alkaloids bear a superficial resemblance to nicotine in their actions on certain smooth-muscle preparations, they do not in fact elicit the type of ganglion response given by nicotine on the tracheal chain.

The remaining drugs tested on guinea-pig trachea, which possess ganglion-stimulant properties in other situations such as the superior cervical ganglion (Burn & Dale, 1914-15; Feldberg & Vartiainen, 1935; Ambache, 1949), were tetramethylammonium, choline, and barium; with none could nicotinic responses be demonstrated. Bronchoconstrictions due to tetramethylammonium were not antagonized by hexamethonium (up to 500  $\mu\text{g}/\text{ml}$ ), but were abolished by atropine 1  $\mu\text{g}/\text{ml}$ . The bronchoconstrictor responses of the trachea to choline were unaffected by hexamethonium (up to 500  $\mu\text{g}/\text{ml}$ ); those to barium were unaffected by hexamethonium, in concentrations of 1 and 10  $\mu\text{g}/\text{ml}$ . It does not necessarily follow that these substances are incapable of nicotinic action on bronchial ganglion cells. Since they can all excite smooth muscle directly, ganglionic excitation would only show itself clearly if this were more readily achieved than smooth muscle stimulation, since in these experiments both ganglion cells and smooth muscle are exposed to the drugs.

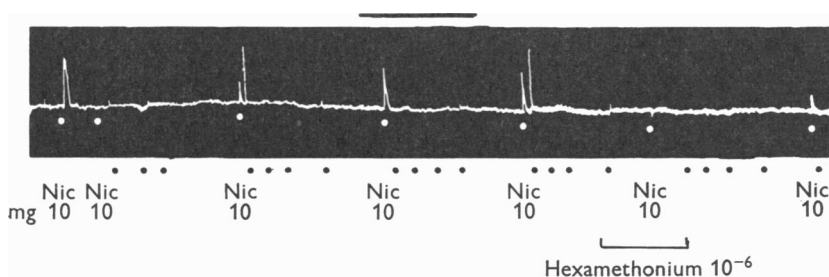


Fig. 9. Cat tracheal ring. Response to nicotine, normally, in the presence of a previous dose or in the presence of hexamethonium  $10^{-6}$ .

#### *Action of nicotine on cat trachea*

Under our experimental conditions, cat tracheal rings, unlike guinea-pig rings, have no resting tone. Whereas guinea-pig rings are immediately relaxed by sympathomimetic amines and by drugs such as caffeine and papaverine, bronchodilator drugs produce no response on the cat trachea unless the preparation has previously been made to contract by some bronchoconstrictor drug.

It was therefore not surprising to find that the primary response of cat trachea to nicotine was not bronchodilatation but a contraction resembling in all respects that seen after physostigmine in the guinea-pig preparation. With the majority of cat tracheal rings nicotine in the range of concentrations

20–400  $\mu\text{g/ml}$ . produced small transient contractions (Fig. 9). After the contraction the trachea was unresponsive to further doses of nicotine until the bath had been repeatedly washed out (Fig. 9). Repeatable and graded responses could be obtained at intervals of about 45 min.

*Nature of the bronchoconstrictor response of cat trachea to nicotine.* The bronchoconstrictor response was greatly potentiated by physostigmine 0.1 and 1  $\mu\text{g/ml}$ . (Fig. 10a), and typical responses to nicotine occurred in those preparations which had previously been unresponsive (Fig. 10b). Hexamethonium 1  $\mu\text{g/ml}$ ., cocaine 1  $\mu\text{g/ml}$ ., or atropine 1  $\mu\text{g/ml}$ . all antagonized the response. Fig. 10b shows also that these concentrations of hexamethonium were specific and had no atropine-like action, since the equivalent responses to acetylcholine were unaffected; atropine, as would be expected, blocked the actions of both nicotine and acetylcholine.

The bronchoconstrictor responses of the isolated cat's tracheal ring to nicotine have, therefore, the characteristics of a cholinergic ganglion-cell response, since they are antagonized: (1) by the presence of a previous dose of nicotine; (2) by hexamethonium; (3) by cocaine and (4) by atropine; but are (5) potentiated by eserine.

*Bronchodilator response to nicotine.* It seemed possible that the failure to observe bronchodilator responses to nicotine on the cat trachea was simply due to the absence of tone in this preparation. The response to nicotine was therefore re-examined on chains which were maintained in a partly contracted state by the presence of pilocarpine 0.25  $\mu\text{g/ml}$ . It was found that under these conditions 20–50  $\mu\text{g/ml}$ . of nicotine gave transient relaxations resembling closely the responses of the guinea-pig trachea to similar doses (Fig. 11). The response to nicotine was blocked by the presence of the previous dose of nicotine left in the bath and by hexamethonium 10  $\mu\text{g/ml}$ . It therefore seemed likely that these responses were mediated by adrenergic ganglion cells, but the responses were not affected by ergotamine 5  $\mu\text{g/ml}$ .

We have concluded that when the cat trachea is partly contracted its primary response to nicotine resembles in most respects that of the guinea-pig trachea. On both preparations the majority of ganglion cells responding to nicotine appear to be adrenergic, producing bronchodilatation, but the presence of cholinergic ganglion cells, producing a bronchoconstriction, can be demonstrated either directly or in the presence of physostigmine.

#### *Action of nicotine on isolated guinea-pig primary bronchi*

The preceding analysis of the responses of guinea-pig and cat trachea to nicotine provided the basis for interpretation of the more complicated response of isolated chain preparations made from the main bronchi of the guinea-pig. The responses of this preparation to nicotine 40–80  $\mu\text{g/ml}$ . had three components. All three were greatly reduced or abolished by hexamethonium

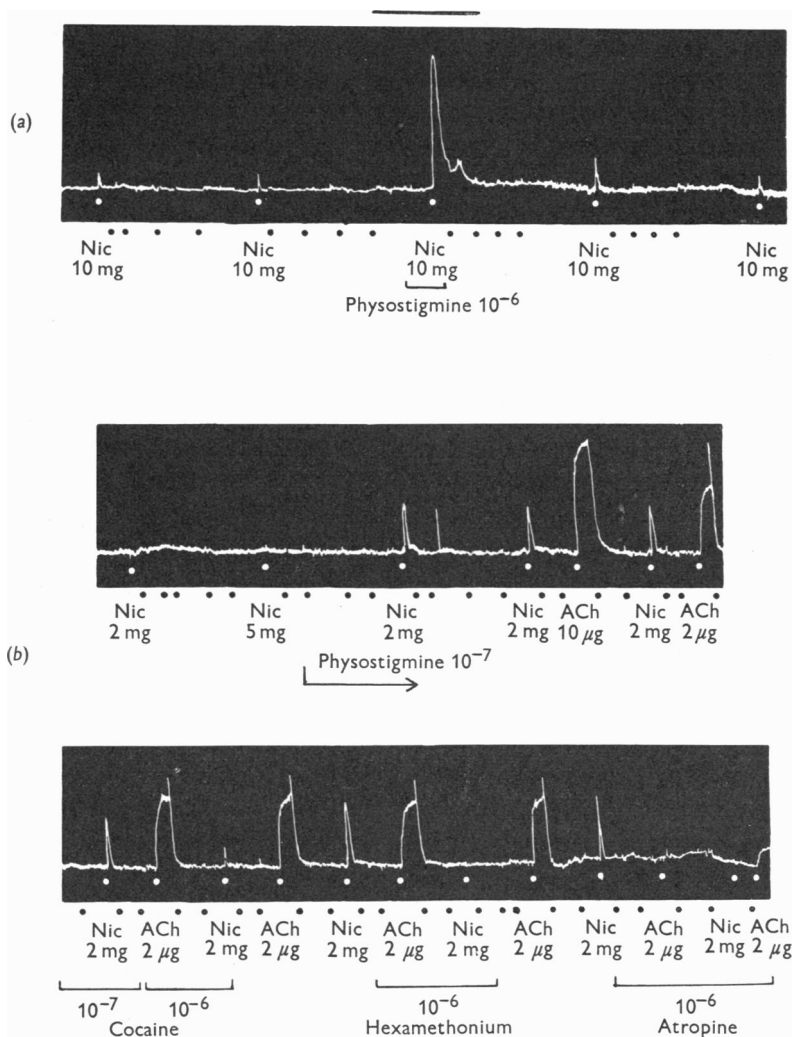


Fig. 10. Cat tracheal ring. (a) Action of nicotine before, during and after physostigmine  $10^{-6}$ ; (b) action of nicotine before and during exposure to physostigmine; abolition of nicotine action by cocaine and by hexamethonium, and of the actions of both nicotine and acetylcholine by atropine.

1  $\mu$ g/ml., or by cocaine 1  $\mu$ g/ml. and were therefore presumed to be due to the action of nicotine on ganglion cells in the tissue.

The first component was a rapid transient contraction readily abolished by atropine 0.2 or 1.0  $\mu$ g/ml. (Fig. 12a, b), and potentiated by physostigmine, 0.1  $\mu$ g/ml. (Fig. 12c). It therefore possessed the pharmacological properties of a cholinergic ganglion-cell response. This transient bronchoconstriction



sometimes waned with repeated responses and then merged with the second component of the nicotine response.

The second component of the response of the guinea-pig's primary bronchus to nicotine consisted of a long-lasting bronchoconstriction, shown in Fig. 12*a*. This part of the response was unaffected by specific concentrations of atropine (Fig. 12*a, b*), though higher concentrations of atropine, up to 5  $\mu\text{g}/\text{ml}$ ., could reduce it somewhat. This is not surprising, since the effect was sensitive to ganglion-blocking drugs, and it is known that high concentrations of atropine

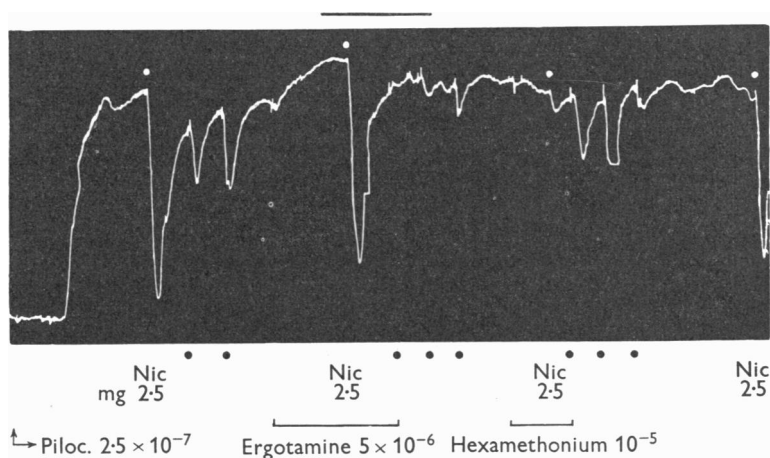


Fig. 11. Cat tracheal ring; tone raised by pilocarpine throughout. Bronchodilator action of nicotine, resistant to ergotamine but greatly reduced by hexamethonium.

have some ganglion-blocking activity. The relative resistance of the second component to atropine suggested that this part of the nicotine response was not cholinergic in nature. This was confirmed by finding that physostigmine, which potentiated the first component markedly, left the second component quite unaffected.

Since the second component of the nicotine response appeared not to be cholinergic in nature, we tested the possibility that histamine might be the post-ganglionic mediator concerned. Figure 13*a* shows such an experiment. It will be seen that the nicotine response displayed the second component fairly well; alternate responses were to histamine. On introducing mepyramine into the Ringer's solution the nicotine response was quite unaffected, although the effects of histamine were abolished. It was found that much higher concentrations of mepyramine, such as might be expected to exhibit local anaesthetic action, would cause some reduction of the nicotine effect, but these concentrations were one thousand times greater than is necessary to reduce the effect of histamine. Further, the specific histamine potentiator  $B_1$ -pyrimidine

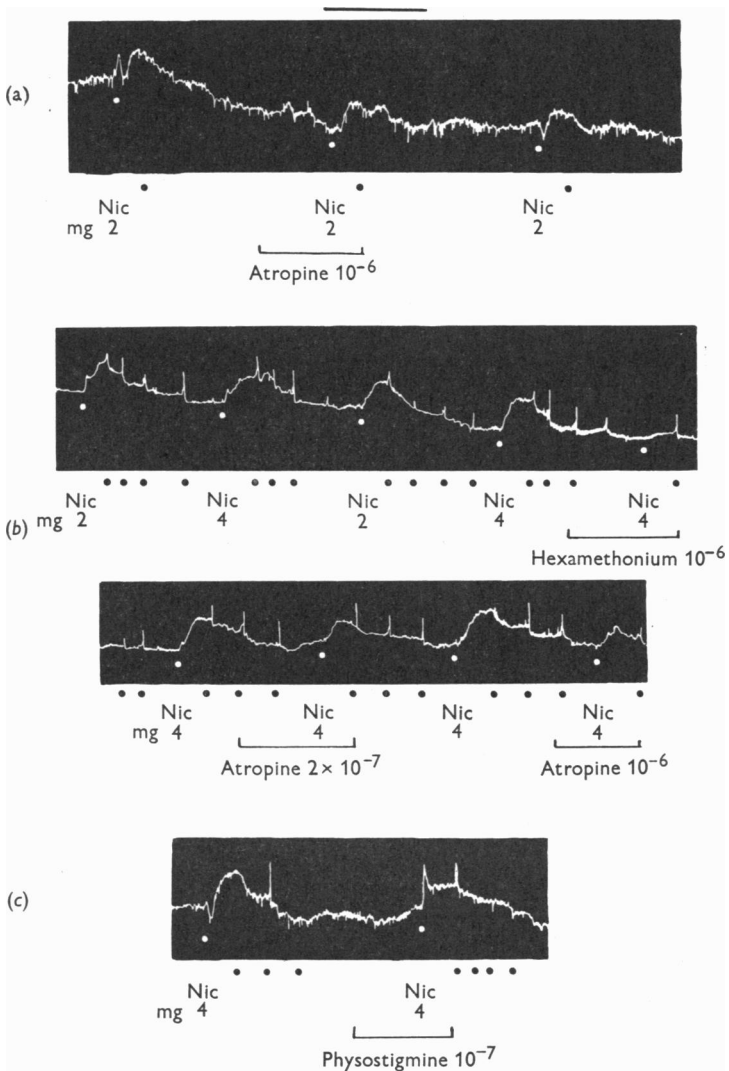


Fig. 12. Guinea-pig primary bronchus. (a) Response to nicotine, and abolition by atropine of initial bronchoconstriction only—after atropine is washed out initial bronchodilatation occurs; (b) response to nicotine, showing its abolition by hexamethonium and the resistance of the slow component of the response to atropine; (c) response to nicotine with initial bronchodilatation; action of physostigmine, showing reversal of the initial response without change of the slow response.

(Arunlakshana, Mongar & Schild, 1954) did not affect the second component of the nicotine response although it strongly potentiated an equivalent histamine response (Fig. 13*b*).

The remaining component of the response of guinea-pig primary bronchi to nicotine, a rapid transient relaxation, was only demonstrable after the first component had been removed by atropine (Figs. 11*a*, 12*a*, *c*). It was blocked by hexamethonium or cocaine. It was not analysed further, but so far as it

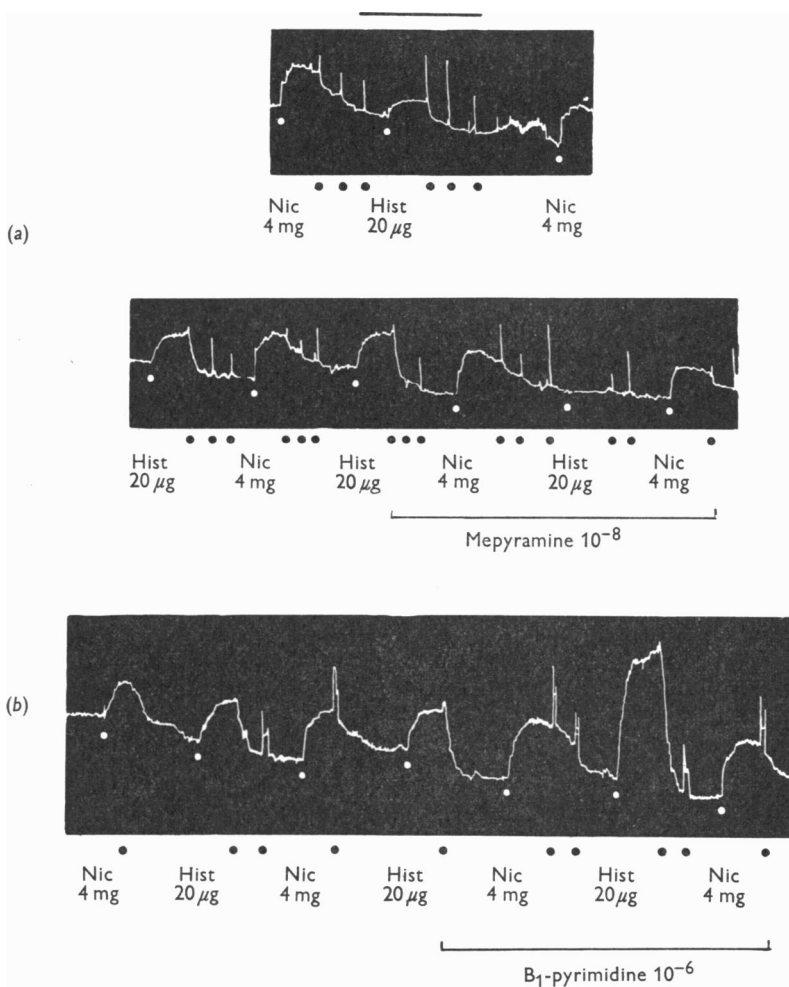


Fig. 13. Guinea-pig primary bronchi. Response to nicotine and to histamine, showing resistance of nicotine slow bronchoconstriction (a) to mepyramine, and (b) to B<sub>1</sub>-pyrimidine.

was studied it resembled the bronchodilator response of the guinea-pig trachea to nicotine, or of the cat trachea after exposure to pilocarpine. This component therefore had the characteristics of a response of adrenergic ganglia.

Thus while the two rapid, transient components of the response to nicotine by the guinea-pig primary bronchus can be readily interpreted as responses

mediated by cholinergic and adrenergic ganglion cells respectively, the prolonged bronchoconstrictor component of the response remains unexplained. This bronchoconstriction has the characteristics of a ganglion-cell response, since it is abolished by hexamethonium and cocaine; but it resists atropine, physostigmine, mepyramine, and  $B_1$ -pyrimidine, and is therefore presumably not mediated by acetylcholine or by histamine.

#### *Other bronchial muscle preparations*

*Guinea-pig.* Experiments were performed using guinea-pig trachea or bronchi suspended longitudinally. With trachea the responses were small and were the inverse of those obtained with tracheal chains prepared in the usual way. Thus histamine, acetylcholine, and barium chloride caused the trachea to lengthen, whereas adrenaline made it shorten slightly. It was concluded that since the tracheal muscle is nearly all circular the longitudinal movement recorded was merely a secondary effect of the circular contraction or relaxation.

Guinea-pig main bronchi, consisting of primary and secondary divisions in continuity, were also tested longitudinally. The muscle here is known to form a network, so that any contraction would be expected to have a longitudinal component. The preparations gave small contractions to histamine, acetylcholine, tetramethylammonium, and barium. But the preparations had little spontaneous tone, and relaxations were not obtained with adrenaline. The responses were always small, and nicotine was inactive. We concluded that these longitudinal preparations offered no advantage over the ordinary chain preparations, and they were not studied any further.

Experiments were also made on tracheal chains prepared from different parts of the trachea. It was found that nicotine was often less active in producing relaxation of the lower third of the trachea than of the upper part, whereas the sensitivity to the sympathomimetic amines was more or less uniform. The responses of the lower third of the trachea, indeed, on occasion seemed to resemble those of primary bronchial chains, consisting of a rapid diphasic response followed by a slow contracture.

On one occasion a preparation of the secondary bronchi, from a large guinea-pig, was tested. The bronchi apparently contained very little muscle, since only a small contraction resulted from the application of a large dose of acetylcholine to the bath. Responses to nicotine in this experiment were small and of doubtful significance.

*Air-perfused guinea-pig lungs.* A preparation of the type described by Arunlakshana & Schild (1950) was used, the pulmonary circulation being perfused with Krebs-Henseleit solution, the bronchial tree with a constant flow of air. The pressure developed in the trachea was recorded with a small mercury manometer, and provided a measure of the bronchial diameter. Under these conditions the bronchi have little or no tone and adrenaline was

inactive. After the perfusion had been set up for 1–2 hr, nicotine injected into the pulmonary artery in a dose of 0.5–1 mg caused a transient bronchoconstriction, which was prevented by previously administered hexamethonium.

*Cat primary and secondary bronchial chains.* These preparations gave responses similar to those of the cat trachea. Nicotine was inactive on the resting preparation, but in the presence of physostigmine elicited a transient bronchoconstriction. When the preparation was maintained partly contracted by the addition of pilocarpine, nicotine produced a transient bronchodilatation. The relaxation produced by nicotine was prevented by hexamethonium.

*Human bronchial chains.* Six preparations were made from bronchi of the fourth, fifth and sixth orders, dissected from apparently normal lobes of lungs removed from two patients with bronchial carcinoma. These preparations have some resting tone; they can be stimulated by acetylcholine and relaxed by adrenaline or noradrenaline. The response to acetylcholine has been shown to be increased by physostigmine (Hawkins & Schild, 1951). No clear-cut responses to nicotine were obtained, however, even in the presence of eserine. It is in fact known that as the periphery of the human bronchial tree is approached ganglion cells rapidly become scanty, the post-ganglionic axons becoming progressively longer and more longitudinally placed and hence more likely to be severed in ring preparations.

#### *Other actions of hexamethonium on bronchial muscle*

The usefulness of hexamethonium in studying ganglionic physiology depends on its specificity of action. Although it has so far proved satisfactory in this respect, some additional experiments were made on bronchial muscle to test how far actions other than ganglionic might be present to complicate the interpretation of our experiments on this tissue.

It was noticed that large amounts of hexamethonium (200–800  $\mu\text{g}/\text{ml}$ .) caused a slowly developing rise in tone of guinea-pig tracheal chains. This was a small but regular action, seen both with the bromide and iodide salts. It cannot be regarded as muscarinic in nature, since it was not abolished by atropine (1–10  $\mu\text{g}/\text{ml}$ .); atropine could, however, delay the action somewhat. Large doses of hexamethonium had no effect of their own on cat bronchial muscle.

Hexamethonium concentrations up to 500  $\mu\text{g}/\text{ml}$  did not antagonize the responses of guinea-pig bronchial muscle to acetylcholine; nor, in doses up to 10  $\mu\text{g}/\text{ml}$ ., did it affect responses of guinea-pig or cat bronchial muscle to pilocarpine. This showed both that hexamethonium is virtually devoid of atropinic activity, and also that, when pilocarpine or acetylcholine was used to increase the tone of some preparations before examining the bronchodilator action of nicotine, the rise in tone was not due to nicotine-like ganglionic stimulation.

In addition, hexamethonium has no anti-histamine action. Indeed, in concentrations of 50–100  $\mu\text{g/ml}$ . it augments the histamine response. This might be due to the histamine-potentiating action common to a number of diamines (Arunlakshana *et al.* 1954) or to a subliminal stimulant action by hexamethonium itself. Hexamethonium, up to 400  $\mu\text{g/ml}$ ., does not affect the response of guinea-pig trachea to adrenaline or noradrenaline.

From the estimation of anticholinesterase activity of other methonium compounds (Paton & Zaimis, 1949) it might be expected that hexamethonium would have a feeble anticholinesterase action *in vitro*. On the guinea-pig tracheal chain high doses of hexamethonium (up to 500  $\mu\text{g/ml}$ .), can, if added to the bath shortly before, augment the action of a small dose of acetylcholine. But it was found that the response to choline under the same conditions was equally augmented, and that if the hexamethonium was given 15–20 min before acetylcholine or choline the augmentation did not occur, although during the interval a rise of tone might take place. It is therefore likely that the hexamethonium was not exerting any anticholinesterase action, but rather that its own feeble stimulant effect can, under suitable conditions, sum with that of acetylcholine or choline.

*Hexamethonium and the anaphylactic bronchoconstriction.* Tracheal chains were taken from a guinea-pig sensitized 4 weeks previously by the injection of egg albumin, 100 mg intraperitoneally and a further 100 mg subcutaneously. The responses in the presence of hexamethonium 20 and 200  $\mu\text{g/ml}$ . were compared with those of a control preparation. All three preparations gave a sustained maximal contraction in response to the addition of egg albumin (100  $\mu\text{g/ml}$ .) to the bath, and no difference in the responses due to the hexamethonium could be detected.

#### DISCUSSION

Using preparations of isolated tracheal and bronchial muscle from guinea-pig and cat, we have demonstrated a number of responses to nicotine, which have the pharmacological characteristics usually attributed to the effects of ganglion-cell stimulation by nicotine. (a) The responses were transient, the smooth muscle recovering without removal of nicotine from the bath, and in this state remaining refractory to further doses of nicotine. (b) The responses were blocked by very low doses of hexamethonium, by 'nicotinic' alkaloids, and by drugs having a local anaesthetic action. (c) The responses were in general blocked by specific antagonists of the appropriate post-ganglionic chemical transmitters. These reactions conform with the known presence of ganglion cells in the respiratory tract.

But the results also point to the presence in the tissue studied of ganglion cells mediating bronchodilator actions in addition to the familiar bronchoconstrictor action. Caution is necessary, however, in assuming that experi-

ments of this type prove the presence of two types of ganglion cell. Nicotine is known to stimulate nerve endings, including sensory nerves in skin, and baroreceptor and chemoreceptor endings (Coon & Rothman, 1940; Brown & Gray, 1948; Ambache & Robertson, 1952; Douglas, 1952; Düner & Pernow, 1952; Diamond, 1955) so that the possibility exists that it may produce its actions by eliciting a local axon reflex from adrenergic or cholinergic nerves, as well as or in place of ganglionic excitation. A crucial experiment to distinguish axonal from ganglion excitation by added drugs is peculiarly difficult to devise. But the known presence of ganglion cells in the bronchi, combined with the fact that hexamethonium in such low concentrations can reduce the effects of nicotine on bronchial muscle, leads us to believe that its actions are, in these experiments, ganglionic. Reports of the use of hexamethonium in antagonizing nicotine on nerve endings usually refer to concentrations of 100  $\mu\text{g}/\text{ml}$ . or more, although Diamond (1955) states that with the isolated perfused carotid sinus of the cat the minimum concentration of hexamethonium necessary to block the acceleration by threshold doses of nicotine or acetylcholine of the discharge from the pressure receptors varied from 0.1 to 10.0  $\mu\text{g}/\text{ml}$ . Transmission in the superior cervical ganglion is reduced by concentrations of hexamethonium of 1.0  $\mu\text{g}/\text{ml}$ . or less. Since the actions of nicotine on the bronchi in our experiments were antagonized by hexamethonium 0.1  $\mu\text{g}/\text{ml}$ ., we shall assume, in this discussion, that the actions of nicotine are ganglionic; if this proves to be wrong the interpretation we give would require modification.

Macklin (1929), reviewing the somewhat confused literature on the subject, consigns nicotine to a class of drugs which are claimed to be bronchodilator by some, bronchoconstrictor by others, and ineffective by still other authors. In the present work the nature of the bronchial response to nicotine depended on whether or not the preparation had a tone. With guinea-pig trachea, which has considerable tone, or with cat tracheal or bronchial rings maintained in a state of partial contraction by pilocarpine, the primary response to nicotine was dilatation. With preparations which had no resting tone and were hence incapable of dilatation—isolated perfused guinea-pig lungs, cat trachea and bronchi—the primary response to nicotine was bronchoconstriction. On the other hand, the presence of a bronchoconstrictor component to the nicotine response could be demonstrated in all the above preparations by the use of physostigmine. Thus both types of ganglion cell appear to be present in the bronchi of guinea-pigs and cats. The nature of the response to nicotine recorded depends on whether or not the constrictor component is enhanced by eserization, and, if our deductions about the functions of the ganglion cells in the bronchi are correct, by the relative proportions of intact adrenergic and cholinergic ganglion cells present.

The fact that we were unable to elicit nicotine responses in preparations of

guinea-pig secondary bronchi or of the smaller human bronchi does not preclude the presence of ganglion cells or cells of similar type in these preparations. It does suggest the absence of entire cells—soma, axon and ending—in the rings we cut. It is known from anatomical studies that the ganglionic elements are disposed more and more longitudinally as the bronchial tree is descended; ganglion cells are rarely found beyond the proximal ends of third-order bronchi in human lung, though the post-ganglionic fibres continue down to the muscle of the smallest bronchioles (Larsell & Dow, 1933). In cutting rings for the preparations of smaller bronchi the axons of such ganglion cells would be severed.

If there are bronchodilator ganglion cells in the bronchial walls, these may on one hand be aberrant sympathetic ganglia and would be brought into play during a sympathetic discharge. On the other hand it may be that they, together with bronchoconstrictor ganglion cells, receive a preganglionic supply from the vagus nerve; this would provide means whereby vagal stimulation could produce bronchodilatation as well as bronchoconstriction. It is then possible that the vagus maintains the balance of resting bronchomotor tone by playing on both types of ganglion cell. Such alternative routes of bronchial control could offer a physiological advantage to the body. The route through the sympathetic nerves could be called into play as part of a generalized sympatho-adrenal discharge. But if a bronchodilatation independent of general sympathetic activity were required, the vagal pathway with its selective action would be more appropriate.

#### *The nature of the post-ganglionic transmitters*

In the experiments on the bronchodilator effect of nicotine on guinea-pig trachea it was demonstrated that the response was antagonized by ergotoxine, ergotamine and dihydroergotamine. Quantitatively, we found that responses to nicotine were antagonized by approximately the same concentration of ergot derivative as that required to antagonize similar responses to adrenaline or to isoprenaline; but noradrenaline effects were antagonized only by considerably larger doses of the alkaloid. This indicates that the post-ganglionic transmitter at the bronchodilator endings in the guinea-pig trachea resembles adrenaline or isoprenaline rather than noradrenaline.

The second response analysed, the transient bronchoconstriction to nicotine, was always abolished by low concentrations of atropine and potentiated by small doses of physostigmine. Transmission at these post-ganglionic endings must be attributed to the muscarinic action of acetylcholine.

The preparations of guinea-pig primary bronchi used showed a more complicated response to nicotine, consisting of three components. The first component was a typical cholinergic bronchoconstrictor response. When this had been abolished by atropine, it was replaced by a transient bronchodilatation



comparable to the adrenergic responses seen with trachea. The remainder of the response is not so easily interpreted. It consisted of a long-lasting slowly developing contracture, which resisted atropine, was not potentiated by eserine and was not affected by mepyramine or  $B_1$ -pyrimidine in sufficient doses to alter equivalent responses to histamine. This contracture was abolished by hexamethonium or cocaine, and there is therefore reason to believe that it was mediated by ganglion cells. Thus it appears to be a ganglionically mediated slow bronchoconstrictor response, atropine-resistant and not involving histamine. This finding is reminiscent of the observations of Henderson & Roepke (1934, 1935) that there is an atropine-resistant component to the motor action of acetylcholine on the bladder, although these authors, as well as Edge (1955) thought it to be mediated via post-ganglionic parasympathetic neurones. Similarly, Sherif (1935) concluded that the hypogastric nerve in the bitch carried cholinergic fibres to the uterus; the response, though atropine-resistant, was enhanced by eserine. It may be that in the bladder, in the uterus, and in guinea-pig primary bronchi, atropine-resistance is a property of the synapse (as suggested by Dale & Gaddum in 1930) and that the transmission is indeed cholinergic. But with guinea-pig primary bronchi the additional fact that the slow contracture is not potentiated by physostigmine favours a transmitter for this part of the bronchoconstrictor response other than acetylcholine. The growing recognition of the biological role played by slow-reacting substances lends some plausibility to this view.

## SUMMARY

1. Nicotine produces a relaxation of the guinea-pig tracheal chain which is abolished by previous treatment with nicotine, hexamethonium, lobeline, coniine, cytisine, sparteine, anagryne or cocaine.
2. This relaxant action could be antagonized by ergotoxine, ergotamine or dihydroergotamine. The activity of these alkaloids against nicotine was comparable to that against adrenaline or isoprenaline; but much higher doses of the antagonists were needed to block the relaxant action of noradrenaline.
3. Atropine or mepyramine in doses specifically effective against acetylcholine or histamine did not reduce this effect of nicotine.
4. It is suggested that nicotine can excite adrenergic ganglion cells in the tracheal wall, and that these cells release a sympathin resembling adrenaline or isoprenaline rather than noradrenaline.
5. If the tone of a guinea-pig tracheal chain is reduced, or if it is treated with physostigmine, nicotine now produces a bronchoconstriction, abolished by ganglion-blocking agents or by atropine. This is believed to be due to excitation of cholinergic ganglion cells.
6. With cat tracheal rings, which have little normal tone, the first action of nicotine is bronchoconstrictor. If the tone is raised with pilocarpine,

bronchodilator responses to nicotine occur. These reactions have the properties of reactions by cholinergic and adrenergic ganglia respectively.

7. Guinea-pig primary bronchi exhibit a third response to nicotine, a rise in tone of slow onset, long-lasting, resistant to atropine or eserine, unaffected by mepyramine or  $B_1$ -pyrimidine, but abolished by hexamethonium or cocaine. This suggests a ganglionic response mediated by some substance other than acetylcholine or histamine, possibly a 'slow-reacting substance'.

8. A number of other preparations of tracheal or bronchial muscle were tested, but offered no advantage over the ring preparations.

9. Hexamethonium was found to be free of atropinic, anti-histamine, or anti-adrenaline action on tracheal ring. In high concentrations, it produced itself some rise of tone not abolished by atropine. Hexamethonium does not modify the anaphylactic response of tracheal muscle.

We should like to express our gratitude to Dr H. G. Herxheimer for suggesting the problem which led to this work.

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