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BRONCHOMOTOR RESPONSES TO STIMULATION OF THE STELLATE GANGLIA AND TO INJECTION OF ACETYLCHOLINE IN ISOLATED PERFUSED GUINEA-PIG LUNGS

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It has previously been reported [Hebb, 1939] that the bronchoconstrictor response to acetylcholine or to stimulation of the stellate ganglia can be reduced or suppressed by administration of ergotoxine. Investigation of this problem has since been continued, and it is now proposed to give a full account of the results obtained in the entire series of experiments.

Methods

Forty-three experiments were performed on the isolated perfused lungs of guinea-pigs bred from the same strain and fed on a uniform diet. The perfusion technique described by Dale & Narayana [1935], Daly, Peat & Schild [1935] and by Petrovskaia [1939] has been adopted. In certain respects the procedure has been modified in order to prevent the early onset of "lung rigidity", a condition to which the perfused guinea-pig lungs are extremely susceptible. To all save six animals, adrenaline was given by subcutaneous injection an hour before death. Immediately after each animal had been killed (by a blow on the head) tracheotomy was performed and positive pressure ventilation begun. Cannulae were tied into the pulmonary artery and left auricle. Then the pulmonary blood vessels were washed through with perfusion fluid, the heart ventricles were fixed into a clamp, and perfusion was begun. During the experiment the animal was kept in a closed chamber (temperature 38–40° C.), where the lungs were maintained under positive pressure ventilation by means of a micro-pump similar to that described by Daly [1937]. The intrapulmonary pressure during a short period of the inflationary stage of each respiratory cycle was recorded on the kymograph by a water pressure-volume recorder.

Two kinds of perfusion fluid have been used: (1) low calcium hypertonic Tyrode solution recommended by Daly *et al.* [1935], and (2) heparinized guinea-pig's blood diluted with one or two volumes of 0·9 % sodium chloride solution. The lungs were perfused at constant pressure by means of either one of two systems:

(1) "Open" perfusion in which the fluid was not recirculated. Tyrode solution was continuously supplied from a flask (37–38° C.) connected directly to the pulmonary arterial cannula, the perfusion pressure being determined by the relative levels of the cannula and the flask.

(2) "Closed" perfusion in which a given volume (20–25 c.c.) of either Tyrode solution or dilute heparinized blood was recirculated by a micro-perfusion blood pump [Daly, 1937] which was adapted so that the inflow into the pulmonary artery was maintained at constant pressure (for description, see Petrovskaia [1939]).

The venous outflow rate was measured in all experiments by means of a drop recorder.

Drugs were administered either by injection into the pulmonary arterial cannula or by adding them in known concentrations to the perfusion reservoir. The following have been used in this investigation: acetylcholine (Roche Products), adrenaline with 0·5 % chloretone (Parke Davis), atropine hydrochloride (B.D.H.), ergotoxine ethanesulphonate (B.D.H.), heparin (Jorpes 1·0 % solution), nicotine (B.D.H.) and physostigmine (B.D.H.).

The stellate ganglia were exposed from the ventro-lateral aspects. Each ganglion was freed from its lateral connexions without disturbing its connexion with the rest of the sympathetic chain (except in some control experiments in which the chain was cut below the level of the stellate ganglion) and without injury to the tissue on its medial border. Then, while the ganglion was lifted slightly by means of a blunt hook inserted from the lateral side, shielded electrodes were slipped into place so that their tips came into contact with the ganglion but did not touch any other structure. For the electrical stimulations a Palmer induction coil was used. Each stimulation was applied for 2 or 3 sec.

Results

(1) *Pulmonary responses to stimulation of the stellate ganglia*

Broncho- and vaso-motor responses to electrical stimulation of the stellate ganglia were observed in twenty-five experiments performed on isolated perfused lungs of guinea-pigs. In each experiment it was found

that such stimulation produced bronchoconstriction. This response was marked by a sharp rise in intrapulmonary pressure which, beginning with the onset of the stimulus, usually reached its maximum within 30 sec. and then began slowly to subside (Fig. 1 B). The rise in intrapulmonary pressure, denoting bronchoconstriction, occurred both with separate and

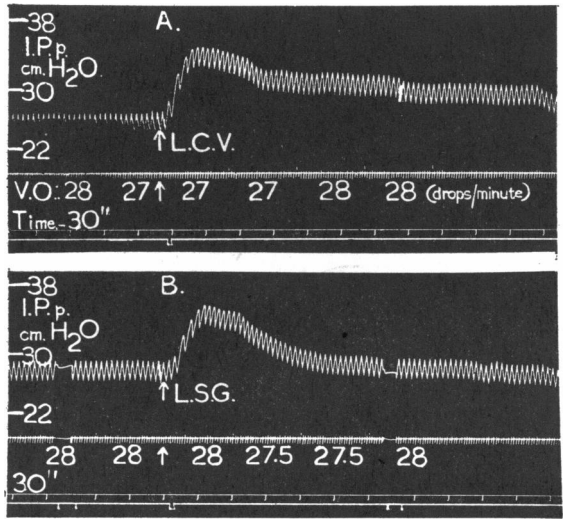

Fig. 1. 13. vi. 39. Guinea-pig 72. ♀; 650 g. Pretreated with adrenaline (0·25 mg. subcutaneously). Lungs perfused with hypertonic glucose-Tyrode solution in closed circulation at initial pressure of +5 cm. perfusate. Reading from top to bottom the four tracings are: the intrapulmonary pressure (I.P.p.); venous outflow rate (V.O.), as registered by the drop recorder (the figures given represent the number of drops per minute); time signal set at 30 sec.; and the signal line. At A (12.03 p.m.) the peripheral end of the cut left cervical vagus was stimulated for 3 sec. with the secondary coil set at 7 cm. At B (12.12 p.m.) the left stellate ganglion was stimulated for 3 seconds at coil distance 7 cm. (No other stimulus was applied between A and B.)

simultaneous stimulation of the right and left ganglia and with stimulation of the thoracic sympathetic chain immediately below the lower pole of the stellate ganglion.

These results are in agreement with those of Binger, Gaarde & Markowitz [1931], who, in a study of reflex bronchomotor phenomena in the guinea-pig, came to the conclusion that only a part of the efferent bronchoconstrictor nerves supplying the lungs are carried by the vagi, and suggest that the remainder are derived from the thoracic sympathetic nerves. The observations cited above are evidence in favour of the correctness of this assumption. Moreover, it has been shown in experi-

ments such as that illustrated in Fig. 1, that the bronchoconstriction produced by stimulation of the stellate ganglia is of the same order as that produced by stimulation of the peripheral ends of the cut cervical vagi. In other experiments of a preliminary nature, evidence was obtained indicating that at least some of the bronchoconstrictor fibres excited by stimulation of the stellate ganglia proceed to the ganglia from the upper dorsal region of the spinal cord and thence are distributed to the lungs. The peripheral distribution of these fibres has not been studied.

At this point the problem of immediate interest was to determine whether the sympathetic bronchoconstrictor nerve fibres are cholinergic or adrenergic. In this connexion it is worth noting that Thornton [1939] has obtained evidence indicating that in the guinea-pig the bronchoconstrictor fibres of the vagi nerves are cholinergic, while Petrovskaia's [1939] experimental results are not incompatible with the thesis that at least some of the same group of nerve fibres are adrenergic. In the present study, the problem of the sympathetic bronchoconstrictor nerves has been attacked by studying the effects produced by various drugs, including eserine, atropine, ergotoxine, nicotine and adrenaline, on the bronchomotor responses to stimulation of the stellate ganglia. The results of these pharmacological tests were as follows.

Comparison of the pulmonary responses to stimulations of the stellate ganglia applied before and after administration of eserine (perfusion concentrations = 1 : 100,000 to 1 : 500,000) showed that the bronchoconstrictor responses during eserine perfusion were either greater in intensity (seven experiments) or longer in duration (six experiments) than in the control observations. In four experiments, one of which is illustrated in Fig. 2, these two effects were combined. In Fig. 2 it will be seen that the stimuli applied at D and at G were of the same strength but that the second stimulus applied during eserine perfusion produced bronchoconstriction which was 50 % greater than that registered at D. It should be added, since it is not clearly shown in the figure, that the contraction period at G was three times longer than the contraction period at D. The potentiation here demonstrated derives additional significance from the fact that previous to the administration of eserine the preparation had exhibited a steady loss in sensitivity, as shown by the diminishing responses to successive stimuli.

Atropine usually produced a fall in intrapulmonary pressure. In seven preparations where the action of the drug was studied, it was found that atropine (in perfusion concentrations of 1 : 2000 to 1 : 70,000) either diminished, or suppressed, or reversed the bronchoconstriction

which was normally produced by stimulation of the thoracic sympathetic nerves. Suppression of the response was the most frequent sequel to administration of this drug (see Figs. 3, 4). In certain of the atropinized animals, however, stimulation of the stellate ganglia produced weak bronchodilatation.

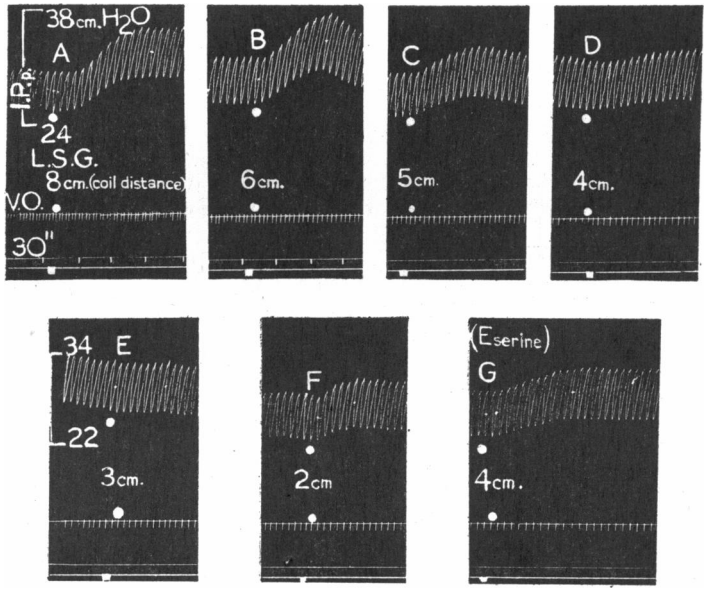

Fig. 2. 21. xi. 38. Guinea-pig 47. ♂; 900 g. Pretreated with adrenaline (0·25 mg. subcutaneously) and heparin (9 mg. intraperitoneally). Lungs perfused with hypertonic Tyrode in open perfusion system at initial pressure of +6 cm. perfusate. The tracings are as in Fig. 1. A–F (3.40–4·05 p.m.), stimulation of the left stellate ganglion at the coil distances shown during Tyrode perfusion. G, 4·13 p.m., stimulation of the left stellate ganglion during eserine-Tyrode perfusion (1 : 500,000).

Administration of ergotoxine was found to have effects similar to those produced by atropine in that it reduced or suppressed bronchoconstrictor responses to sympathetic nerve stimulations (see Fig. 3). Doses of ergotoxine varying from 300μg. to 1 mg. (perfusion concentrations = 1 : 20,000 to 1 : 100,000) produced suppression of the response for periods varying from 25 min. to 1 or 2 hr. (nine experiments).

In view of the observation made in the above experiments that the bronchoconstrictor response could be suppressed either by atropine or ergotoxine, it was not at all clear what type of nerve fibres were involved. It was hoped that some light on the problem might be gained by a study

of the responses of nicotinized lungs. In seven experiments it was found that initial injections of nicotine (50–100 μg.) produced marked bronchoconstriction and rendered the lungs insensitive to further injections of the same drug. However, the lungs continued to respond normally to stimulation of the stellate ganglia, the stimulations producing in the

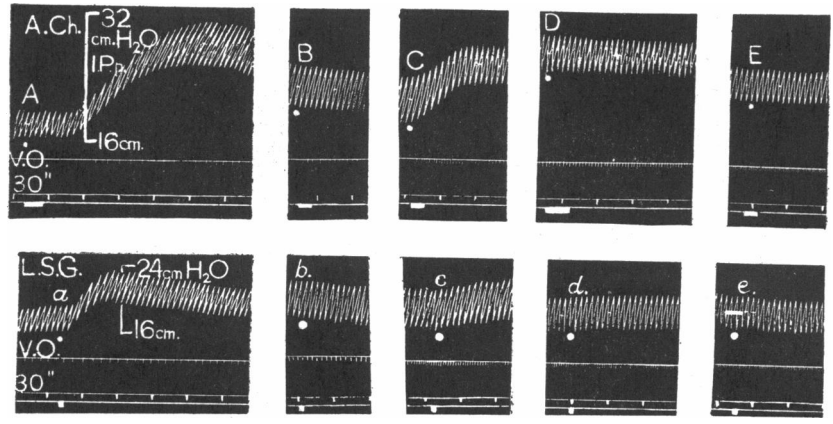

Fig. 3. 24. iv. 39. Guinea-pig 63. ♀; 460 g. Pretreated with adrenaline (0·25 mg. subcutaneously) and atropine (0·1 mg. subcutaneously). Lungs perfused with hypertonic glucose-Tyrode in closed circulation at initial perfusion pressure of +3 cm. perfusate. Tracings are as in Fig. 1. The order of events in this experiment was as follows:

A. 12.44 p.m. 20 μg. ACh.
12.55 p.m. 300 μg. *ergotoxine* injected.

B. 1.06 p.m. 20 μg ACh

C. 1.55 p.m. 20 μg. ACh.
2.12 p.m. 200 μg. *atropine* injected.

D. 2.23 p.m. 20 μg. ACh.
2·32 p.m. 100 μg. *atropine* injected.

E. 2.40 p.m. 20 μg. ACh.

a. 12.35 p.m. left stellate ganglion, coil distance 1 cm.

b. 1.09 p.m. left stellate ganglion, coil distance 1 cm.

c. 1.47 p.m. left stellate ganglion, coil distance 1 cm.

d. 2.26 p.m. left stellate ganglion, coil distance 1 cm.

e. 2.37 p.m. left stellate ganglion, coil distance 1 cm.

(Each stimulation of the stellate ganglion lasted approximately 3 sec.)

nicotinized preparations bronchoconstriction of the same order as that observed in the untreated lungs (see Fig. 6). It seemed clear, therefore, that at least a majority of the sympathetic bronchoconstrictor fibres do not relay within the area supplied by the pulmonary perfusion system. In view of this conclusion, the earlier finding that eserine potentiates the bronchoconstrictor response points to the probability that cholinergic post-ganglionic neurones are involved in that response.

There were other possibilities to be considered as well. Petrovskaia [1939] observed in some of her experiments on guinea-pigs that, within the same experimental period, stimulation of the cervical vagosympathetic nerves, and injection of adrenaline separately, gave rise to bronchoconstriction; and that both effects could be reversed or abolished by ergotoxine. Accordingly, she suggested that there were adrenergic bronchoconstrictor fibres in the cervical vagosympathetic nerve bundles.

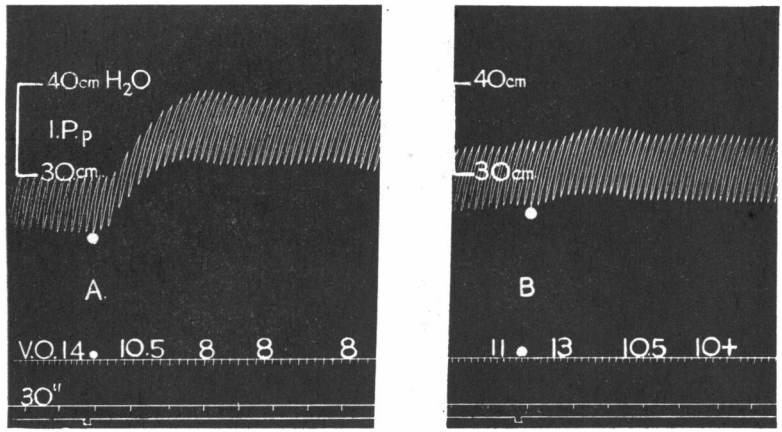

Fig. 4. 7. xi. 38. Guinea-pig 44. ♂; 700 g. Pretreated with adrenaline (0.25 mg. subcutaneously). Perfused with hypertonic Tyrode in open perfusion system at initial perfusion pressure of +5 cm. perfusate. Tracings as in Fig. 1. A, 1.37 p.m., stimulation of left stellate ganglion at coil distance 5 cm. for 3 seconds during eserine-Tyrode (1 : 500,000) perfusion. B, 1.56 p.m., the same stimulus repeated during atropine-Tyrode (1 : 10,000) perfusion. The perfusion fluid was changed from eserine to atropine-Tyrode at 1.47 p.m.

A similar argument might apply to the sympathetic bronchoconstrictor fibres but for one circumstance: it has been observed in the present experiments that, at a time when stimulation of the stellate ganglia caused bronchoconstriction, injections of adrenaline (1–20μg.) invariably produced bronchodilatation. Thus the results obtained with adrenaline injections lent no support to the view that the sympathetic bronchoconstrictor nerves may be adrenergic.

Injected adrenaline in many preparations produced a marked change in the responses of the lungs to subsequent stimulations of the stellate ganglia. Small doses (1–10μg.) injected into the pulmonary artery usually produced depression of the bronchoconstrictor effect for a short period (up to 20 min.) during which nerve stimulations either produced

no bronchomotor change or caused weak bronchodilatation as is illustrated in Fig. 5. When the lungs were perfused with adrenaline in larger concentrations (1–4μg.), as was the case in four experiments, the largest

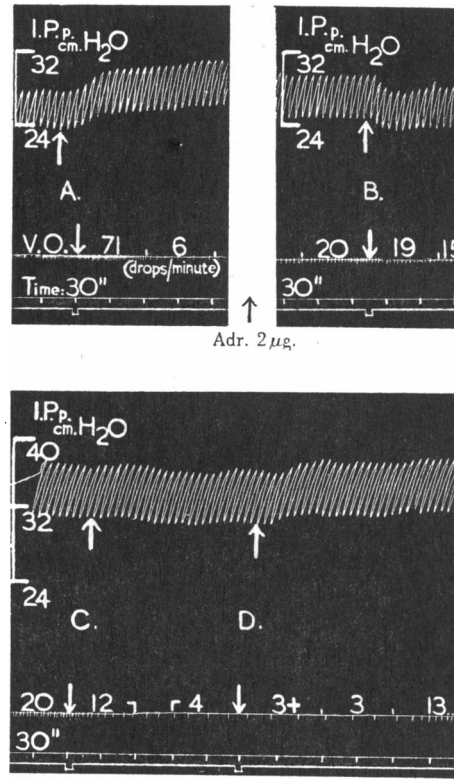

Fig. 5. 18. xi. 38. Guinea-pig 46. ♂; 817 g. Pretreated with adrenaline (0·25 mg. subcutaneously). Lungs perfused with hypertonic Tyrode in open perfusion system at initial pressure of +6 cm. perfusate. The tracings are in the same order as in Fig. 1. A, 12.26 p.m. Stimulation of both stellate ganglia, coil distance 8 cm. B, 12.45 p.m. Stimulation of both stellate ganglia, coil distance 7 cm. C, 12.56 p.m. Stimulation of both stellate ganglia, coil distance 5 cm. D, 12.57 p.m. Stimulation of both stellate ganglia, coil distance 5 cm. Between A and B, at 12.40 p.m., 2μg. adrenaline was injected into pulmonary arterial tubing.

bronchoconstrictor responses observed during a period of 2 hr. or more were so slight as to be just perceptible. This is in line with the results of Cordier & Magne [1927], who found that in the guinea-pig administration of adrenaline depressed the activity of the vagal bronchoconstrictor nerve fibres.

In the same study Cordier & Magne found that stimulation of the thoracic sympathetic nerves occasionally produced weak bronchodilatation, this result being regarded by them as evidence that there are sympathetic bronchodilator fibres innervating the guinea-pig lungs. To some extent that view is confirmed by two observations which have

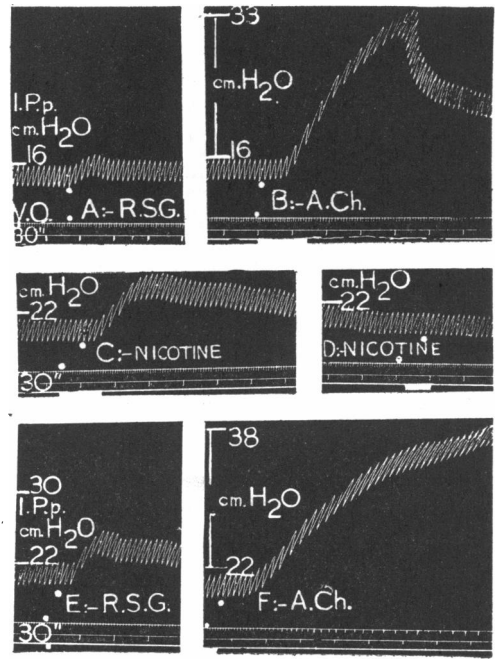

Fig. 6. 20. v. 39. Guinea-pig 69. ♀; 560 g. Pretreated with adrenaline. Perfused with hypertonic glucose-Tyrode in closed circulation at initial perfusion pressure of +4 cm. perfusate. Tracings as in Fig. 1. At A (12.19 p.m.) and E (1.49 p.m.) the right stellate ganglion was stimulated for 3 sec., at coil distances of 5 cm. and 3 cm. respectively. At B (12.26 p.m.) and at F (2.18 p.m.) 10 μg. of ACh. were injected. At C (12.41 p.m.) and at D (12.49 p.m.) 50 μg. of nicotine were injected.

already been mentioned in the present communication: one, that small doses of adrenaline may reverse the normal bronchoconstrictor response so that bronchodilatation occurs instead (cf. Fig. 5A, B); and two, that with injection of atropine a similar reversal occurred in some preparations. It should be added, however, that Cordier & Magne did not find in any of their experiments that stimulation of the sympathetic nerves produced bronchoconstriction. This negative result may perhaps be

explained by the fact that the authors used anaesthetized (urethane) guinea-pigs for their experiments.

In response to stimulation of the thoracic sympathetic in guinea-pigs anaesthetized with nembutal, the only effect on the lungs observed by Dale & Narayana [1935] was vasoconstriction in one out of a total of four experiments. Since the effect was obtained in the absence of any bronchomotor change, the experiment may be regarded as evidence of the occurrence of pulmonary vasoconstrictor fibres in the thoracic sympathetic nerves of the species.

With the methods used in the present experiments for measuring circulatory changes, interpretation of the observations was complicated by the effects of concomitant bronchomotor changes. None the less, a certain amount of valuable information has been obtained by analysis of a total of 311 blood outflow responses which occurred during as many stimulations of the stellate ganglia under a variety of experimental conditions and against a known background of intrapulmonary pressure changes. Of these observations, forty-six were obtained under control conditions, eighty-two after the addition of adrenaline only to the perfusion fluid and the remainder (183) either in eserinized preparations or in preparations treated with atropine or ergotoxine. In many experiments there were sufficient control observations with which to compare the responses obtained after injection of drugs.

In assessing these data, it was frequently found that, in response to stellate ganglion stimulation, the same order of bronchomotor change was not necessarily accompanied by an unvarying venous outflow response. On the other hand, the venous outflow responses might remain the same while the concomitant bronchomotor responses varied widely. Thus it appeared that the bronchial and vascular reactions to the same stimulus, i.e. the excitation of the nerves, were largely independent of each other. This conclusion was substantiated by a further analysis of individual experiments. The relevant facts and inferences about the pulmonary vasomotor responses may be summarized as follows:

Allowance having been made for possible mechanical vasoconstriction produced by a coincident rise in intrapulmonary pressure, it was possible to show that under certain conditions vasoconstriction occurred as the direct result of the nerve stimulations because (1) it was found frequently that with the same order of bronchoconstriction the reduction in blood outflow was at first very marked but subsequently became smaller until it disappeared altogether or remained only just perceptible; and (2) in

sixteen observations (out of a total of thirty-four), where no bronchomotor changes occurred owing to previous injections of drugs, it was observed that marked reductions in venous outflow occurred in response to stimulation of the stellate ganglion.

It was observed that vasodilatation occasionally occurred in response to stimulation of the stellate ganglia. For example, in one experiment a weak stimulus caused vasodilatation while a strong stimulus produced vasoconstriction. In others, vasodilatation was seen to occur after a series of gradually diminishing vasoconstrictor responses, an observation which suggests that vasodilator fibres came into action when the vasoconstrictor fibres were fatigued. With or without administration of drugs, the vasodilator response has been obtained in the absence of any bronchomotor change in eleven out of thirty-four observations. Individual experiments did not give any certain indication of the effects of adrenaline, atropine or ergotoxine on the vascular responses to sympathetic nerve impulses but when assessed in groups it was found that for a given order of bronchomotor change adrenaline did not alter the ratio of the total number of vasodilator to total number of vasoconstrictor responses (shown by comparison with the control groups); both ergotoxine and atropine doubled the percentage occurrence of vasodilator responses.

The foregoing evidence suggests that in the guinea-pig the lungs are innervated by sympathetic vasoconstrictor and -dilator fibres as well as by bronchoconstrictor and -dilator fibres.

(2) *Acetylcholine*

The effects of single injections of acetylcholine (1–100μg.) have been studied in twenty-eight experiments. Confirming the results of previous workers [Dale & Narayana, 1935; Petrovskaia, 1939; Thornton, 1939], it has been found that injections of acetylcholine into the pulmonary circulation cause bronchoconstriction.

The maximal bronchoconstrictor responses elicited by acetylcholine injections in the various experiments, and measured in terms of the positive change in intrapulmonary pressure (I.P.p.) varied for different doses of the drugs as shown in Table I.

Responses obtained in the adrenaline-Tyrode perfused lungs were uniformly less than those obtained in preparations in which either no adrenaline or small amounts (1–10μg.) had been added. This accounts for the discrepancy suggested by the values of the table, that the efficacy increases with doses from 1 to 20μg. and diminishes with doses greater than 20μg. The explanation is that the larger doses (25–100μg.) were

TABLE I

μg. ACh.	I.P.p. change +cm. H_2O	No. of experiments
1	0·8* to 3·8	4
2	0·8 to 10·9	3
5	1·9 to 18·8	3
10	0·6* to 24·4	13
15	15·6	1
20	0·6* to 25·6	6
25	3·8* to 5·0	2
30	2·0*	1
40	1·8*	1
100	5·0*	1

The figures with asterisks represent maximal responses obtained in experiments in which adrenaline had been added to the perfusion fluid (1–4μg./c.c.). I.P.p. = intrapulmonary pressure.

only used after the preparation had been rendered insensitive by adrenaline. Thus the depressant action of adrenaline on the activity of bronchoconstrictor nerves has an analogy in its effect on the bronchial reactions to acetylcholine. Although the actual mechanism of the suppression of these bronchomotor responses is not as yet clear, some of the conditions under which it occurs were demonstrated in experiments similar to the one shown in Fig. 7.

In twelve experiments both acetylcholine and stellate ganglion stimulations were tested under the same conditions and it was observed that the preparations most sensitive to acetylcholine were most sensitive to stimulations of the ganglia.

In an earlier communication [1939] I stated that the action of acetylcholine on the bronchi "is usually prolonged. Whether it is potentiated by eserine there is as yet no evidence on which to decide." This statement was made in reference to my own experiments only, as I should have made clear at the time, since it had already been shown by Thornton [1939] that in the guinea-pig both acetylcholine and vagal bronchoconstriction are potentiated by eserine (1 : 200,000). In recent experiments I have been able to confirm this result.

In the preliminary experiments when testing eserine potentiation of acetylcholine, only Tyrode-perfused preparations were used. With this type of perfusion a slight improvement of acetylcholine bronchoconstriction by means of eserine was observed in one experiment, while in another no effect on the response was observed. These more or less negative results were ascribed to the absence of esterase from the circulating fluid, a view supported by the prolonged acetylcholine-bronchoconstriction and the sluggishness of its recovery even in the non-eserinized preparation. Further support is given to this hypothesis by

more recent experiments on blood-perfused lungs in which recovery from acetylcholine bronchoconstriction was usually rapid and eserine potentia-

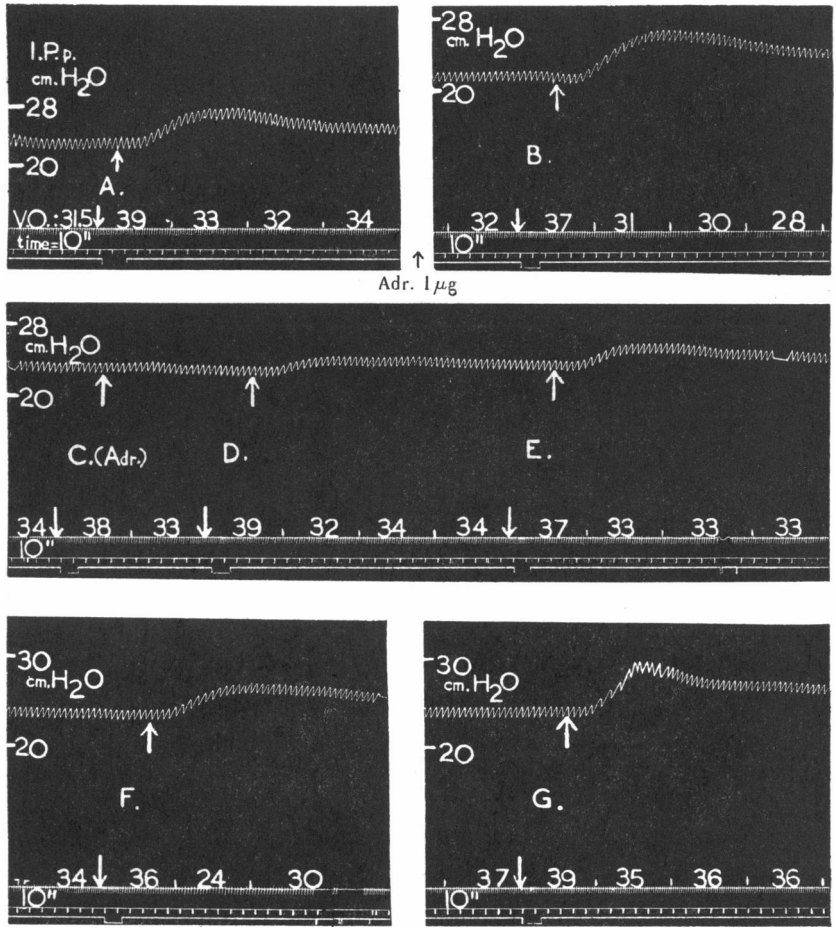

Fig. 7. 18. x. 39. Guinea-pig 85. ♀; 575 g. Pretreated with heparin (10 mg. intraperitoneally) only. Perfused with blood-Tyrode (1 : 1 dilution) in closed circulation at initial perfusion pressure of +5 cm. perfusate. Tracings as in Fig. 1. At A (2.03 p.m.), B (2.25 p.m.), D (2.33 p.m.), E (2.37 p.m.), F (2.45 p.m.) and G (3.17 p.m.) injections of 10 μg. of ACh. were given. Between A and B at 2.20 p.m. 1 μg. of adrenaline was injected; and at C (2.31 p.m.) 10 μg. of adrenaline were injected.

tion was readily demonstrated. In one case the potentiation amounted to about 100 % and in another case (Fig. 8) it was about 450 %. In the

experiment in Fig. 8, an approximately minimal effective dose of acetylcholine, 2μg., produced, in three observations, rises in the intrapulmonary pressure of +0·4, +0·8 (8A) and +0·6 cm. H_2O (8B), in that order. After the administration of eserine, the same amount of acetylcholine produced a pressure change of +3·5 cm. H_2O (8C). In both of the experiments quoted eserine increased not only the degree but also the duration of bronchoconstriction.

The finding of Dale & Narayana [1935] and of Petrovskaia [1939] that atropine abolishes acetylcholine bronchoconstriction has been confirmed in three experiments. The dose of atropine required to produce this effect varied from 100 to 300μg. where the total perfusion volume was about 20 c.c. (recirculated). Suppression by atropine of the bronchomotor

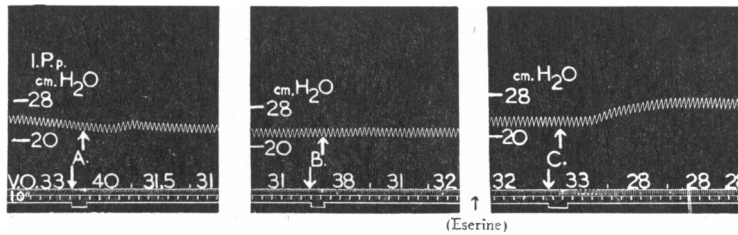

Fig. 8. 1. xi. 39. Guinea-pig 88. ♂; 390 g. Pretreated with heparin (10 mg. intraperitoneally) only. Perfused with blood-Tyrode (1 : 1 dilution) in closed circulation at initial perfusion pressure of +5 cm. perfusate. Tracings as in Fig. 1. At A (3.12 p.m.), B (3.18 p.m.) and C (3.42 p.m.), injections of 2μg. of ACh. were given. Between B and C at 3.22 p.m. 100μg. of eserine were added to the reservoir.

response to stellate ganglion stimulation and acetylcholine was obtained in one experiment in which ergotoxine also suppressed these responses (Fig. 3).

In nicotinized preparations, the bronchial response to acetylcholine was much the same as in the untreated lungs (eight experiments). Compared with control observations the acetylcholine bronchoconstrictor effect was of the same order after nicotine had been injected. The effect of nicotine on the pulmonary responses to acetylcholine and the stellate ganglion is shown in the experiment to which reference has already been made (Fig. 6).

The contraction of the bronchial smooth muscle to acetylcholine, the potentiation of this effect by eserine, and its suppression by atropine were phenomena to be expected in the light of our present knowledge of acetylcholine action, but the reduction or suppression of acetylcholine effects by ergotoxine have been reported only recently [Foggie, 1940].

The results of twenty-four experiments showed that in suitable concentration ergotoxine suppressed acetylcholine bronchoconstriction (see Fig. 3). Recovery of the response from ergotoxine occasionally occurred within 30 min. but was usually more delayed (1–2 hr.). The effective dose of ergotoxine depended partly upon the amount of acetylcholine being given by single injection and partly on circumstances discussed more fully below.

Injection of ergotoxine itself often increased the intrapulmonary pressure level so that a possible explanation of the reduction of the acetylcholine response by ergotoxine was that the latter drug may have increased the tonus of the bronchi so that they were incapable of further contraction. This explanation has been rendered invalid by the observation that after suppression by ergotoxine, the bronchoconstrictor response may recover with repeated injections of acetylcholine, although the intrapulmonary pressure level between injections remains approximately the same. Also it has been found that suppression of the response may continue even when the level has fallen considerably. Finally, in several experiments in which the intrapulmonary pressure was the same after ergotoxine as before, suppression of the acetylcholine response nevertheless occurred.

The doses of ergotoxine varied from 10μg. to 1·5 mg., which correspond to perfusion concentrations of 1 : 2,500,000 to 1 : 1300. The more usual concentrations employed were from 1 : 25,000 to 1 : 50,000. The effectiveness of any given dose depended chiefly upon the sensitivity to acetylcholine originally exhibited by the individual lung preparations. It was also seen that while a given amount of ergotoxine might abolish the bronchoconstriction produced by a given amount of acetylcholine, it did not necessarily suppress the effect of larger doses of acetylcholine. The effective suppressive dose of ergotoxine varied between 300μg. and 1·0 mg. when the dose of acetylcholine was 10–20μg. In a few tests only 1 mg. of ergotoxine was insufficient to suppress the response entirely although it produced a reduction of 50 %.

It was observed that in adrenaline-Tyrode perfused preparations in which the bronchoconstrictor responses to acetylcholine were less than in adrenaline-free preparations, relatively small doses of ergotoxine (300μg.) abolished these responses entirely. However, the action of ergotoxine in suppressing acetylcholine bronchoconstriction was not dependent upon the addition of adrenaline to the pulmonary circulation, since suppression was produced in preparations to which no adrenaline had been previously given either before or after the death of the animal.

From these and other control experiments it became evident that adrenaline and ergotoxine may act synergistically to abolish acetylcholine bronchoconstriction but they can each produce the effect independently of one another.

The effect of ergotoxine on acetylcholine bronchoconstriction was the same in nicotinized preparations as in the untreated lungs (four experiments).

With regard to the pulmonary vascular responses to administration of acetylcholine, little can be added to the results of Petrovskaia [1939] and those of Dale & Narayana [1935]. Like the former author, I have found that the most usual effect of acetylcholine injection was bronchoconstriction associated with a diminished venous outflow. The results of the control observations can be expressed as follows:

I.P.p. + associated with V.O. − in 23 experiments,
I.P.p. + associated with V.O. + in 1 experiment,
I.P.p. + associated with V.O. + and − in 1 experiment,
I.P.p. + associated with V.O. change doubtful in 3 experiments,

where I.P.p + = a rise in intrapulmonary pressure and V.O. + and − = increase and decrease respectively in the venous outflow.

The evidence obtained was not sufficient to decide whether the reduction in venous outflow observed in the majority of experiments was due to the vasoconstrictor action of acetylcholine or was a mechanical effect consequent upon the coincident bronchomotor response. Individual observations indicated that acetylcholine may have acted directly on the pulmonary blood vessels to produce constriction, because the venous outflow reduction was often much greater than would be expected, if it were solely due to the bronchomotor change accompanying it; and because very often in the same experiment the venous outflow responses to acetylcholine injections varied, while the bronchomotor responses remained fairly constant and vice versa.

It has been reported by Dale & Narayana [1935] that perfusion of guinea-pig lungs with adrenaline in a concentration of 1 : 250,000 intensifies the normal vasoconstrictor reaction to acetylcholine. Petrovskaia [1939] was unable to find that adrenaline produced such an effect when given in small single injections (1–2μg.). In this connexion it may be observed in the experiment shown in Fig. 7 that whereas an initial injection of 10μg. of acetylcholine did not reduce the pulmonary venous outflow rate at all, a second injection a few minutes subsequent to the administration of 1μg. of adrenaline did do so to a noticeable degree. The

effect of 10μg. of adrenaline was not so definite. In view of the fact, however, that with a dose of 1μg. the concentration of adrenaline in the pulmonary circulation would be equivalent to the perfusion concentration used by Dale and Narayana, the experiment is interesting as a confirmation of their results.

It should be added that acetylcholine injections have been observed to produce vasoconstriction in the nicotinized as well as in the normal perfused guinea-pig lungs (cf. Fig. 6). Also, as might be expected, the vasoconstrictor response to acetylcholine tended to disappear when atropine or ergotoxine had been added to the perfusate (cf. Fig. 4). Whether or not these effects are attributable to a direct action on the pulmonary blood vessels or are an expression of concomitant bronchial responses cannot be decided on the evidence of the experiments discussed here.

Discussion

The most interesting finding of the experiments which have been described is the observation that the administration of acetylcholine and the excitation of the upper thoracic sympathetic nerves both produce bronchoconstriction in the guinea-pig. A second point is that, so far as the investigation has proceeded, the same conditions have been found to govern the response to each of these two stimuli. In both cases the bronchoconstrictor response is enhanced by eserine, temporarily depressed by adrenaline, and wholly or partly suppressed for a longer period by atropine or ergotoxine. The parallel suggests that the bronchoconstrictor fibres excited by stimulation of the stellate ganglion may be cholinergic. It may appear at first sight somewhat paradoxical that the suppression by ergotoxine of stellate ganglion bronchoconstriction is brought forward as evidence that the response involves the participation of cholinergic nerve fibres, yet the fact that ergotoxine suppresses the bronchoconstrictor activity of acetylcholine is sufficient justification for tentatively adopting this line of reasoning.

No explanation has as yet been found to account for the action of ergotoxine in suppressing acetylcholine bronchoconstriction. Ergotoxine was originally used for the purpose of determining whether or not the sympathetic bronchoconstrictor fibres were adrenergic. It is worth noting in this connexion that Petrovskaia [1939] has suggested that among the cervical vagal and cervical sympathetic fibres there may be some adrenergic bronchoconstrictor fibres, since the bronchoconstrictor effect of cervical vago-sympathetic stimulation may be suppressed or reversed by ergotoxine. My own subsequent experiments revealed, how-

ever, that ergotoxine suppresses the acetylcholine bronchial response as well, so that there are no longer grounds for assuming that ergotoxine acts specifically to prevent only adrenergic nerve motor responses. Nor can it be assumed that ergotoxine will not act to prevent the motor response to cholinergic nerve stimulation.

That these conclusions may have a more general application is suggested first by experiments reported by Foggie [1940], in which she found that ergotoxine and adrenaline reverse the pulmonary vasomotor effect of acetylcholine in the nicotinized perfused lungs of the dog; and secondly by Petrovskaia's observation that vagal bronchoconstriction in the guinea-pig is reversed by ergotoxine; and finally by the experimental results of Brown, McSwiney & Wadge [1930], who have reported that ergotoxine may suppress the motor response of the gastric musculature to stimulation of the sympathetic nerve supply.

The experimental evidence reviewed here appears more surprising in view of the results of Matthes [1930] and of Loewi & Navratil [1926] showing that ergotamine and ergotoxine may enhance the action of acetylcholine by inhibiting the action of esterase. No effect comparable to this was noted either by Petrovskaia or by me in the study of the isolated perfused guinea-pig lungs.

A recent report by Linegar [1940] on the related actions of acetylcholine and ergotamine should also be mentioned here. This worker found that in dogs and cats treated with atropine or with eserine and atropine, ergotamine reverses the pressor action of acetylcholine; and he concludes that ergotamine accomplishes the reversal by increasing the sensitivity of the blood vessels to the vasodilator action of acetylcholine. In so far as the present experiments are concerned, there is as yet no evidence for believing that acetylcholine exerts any dilator action on the bronchi, so that it is unlikely that suppression of the acetylcholine bronchoconstrictor response by ergotoxine depends upon the mechanisms suggested by Linegar [1940] for the systemic blood vessels.

Summary

1. The most usual effect of electrical stimulation of the stellate ganglion on the isolated perfused lungs of the guinea-pig is the production of marked bronchoconstriction. Occasionally, under special conditions, a dilator response also occurs.

2. The bronchoconstrictor response to stellate ganglion stimulation is qualitatively similar to that produced by injection of acetylcholine. Both are potentiated by eserine, temporarily depressed by suitable doses

of adrenaline, and are suppressed wholly or partly by ergotoxine and by atropine.

3. The bronchoconstriction following stimulation of the stellate ganglion or injections of acetylcholine occurs in nicotinized lung preparations as well as in the normal preparations.

4. Some evidence has been given that in addition to bronchoconstrictor and dilator fibres there are vasoconstrictor and dilator fibres reaching the lungs by way of the stellate ganglion.

5. The evidence suggests that acetylcholine may act directly on the pulmonary blood vessels to produce vasoconstriction as distinct from mechanical effects exerted by the coincident changes in the bronchi.

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REFERENCES

Binger, M. W., Gaarde, F. W. & Markowitz, J. [1931]. *Amer. J. Physiol.* **96**, 647.
Brown, G. L., McSwiney, B. A. & Wadge, W. J. [1930]. *J. Physiol.* **70**, 253.
Cordier, D. & Magne, H. [1927]. *Ann. Physiol. Physicochim. biol.* **3**, 486.
Dale, A. S. & Narayana, B. [1935]. *Quart. J. exp. Physiol.* **25**, 85.
Daly, I. de Burgh [1937]. *J. sci. Instrum.* **14**, 276.
Daly, I. de Burgh, Peat, S. & Schild, H. [1935]. *Quart. J. exp. Physiol.* **25**, 33.
Foggie, P. [1940]. *Quart. J. exp. Physiol.* **30**, 13.
Hebb, C. O. [1939]. *J. Physiol.* **96**, 29*P*.
Linegar, C. [1940]. *Amer. J. Physiol.* **129**, 53.
Loewi, O. & Navratil, E. [1926]. *Klin. Wschr.* **5**, 894.
Matthes, K. [1930]. *J. Physiol.* **70**, 338.
Petrovskaia, B. [1939]. *Quart. J. exp. Physiol.* **29**, 121.
Thornton, J. W. [1939]. *J. Physiol.* **96**, 53*P*.