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BRONCHIAL CIRCULATION AND PULMONARY VASOMOTOR NERVE RESPONSES IN ISOLATED PERFUSED LUNGS

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It is well known that pulmonary vasomotor responses to nerve stimulations in isolated lungs of the dog perfused through the pulmonary circulation alone are ephemeral or absent (for literature see Daly, 1958). It was pointed out by Berry, Brailsford & Daly (1931) that failure to obtain clear evidence of pulmonary vasomotor nerve activity in such perfused preparations might be due to an insufficient blood supply to the nerves, which Reisseisen (1822) had shown were supplied by the bronchial arterial system. For this reason they advocated additional perfusion of the bronchial vascular system. This method of perfusion was used by Daly & von Euler (1932), who obtained well marked pulmonary vasomotor responses to nerve stimulations over a period of several hours. Since that time it has been repeatedly observed that such responses are only obtained during bronchial circulation perfusion and are extinguished if this perfusion is interrupted. These observations, however, provided little evidence of the underlying mechanism reponsible for the maintenance of pulmonary vasomotor nerve function by bronchial circulation perfusion. The investigation here described on the lungs of the dog relates to (1) the survival of pulmonary vasomotor nerves during bronchial circulation ischaemia, (2) the bronchial circulation perfusion time required to recover pulmonary vasomotor nerve function after varying periods of ischaemia, (3) the duration of bronchial circulation ischaemia which extinguishes pulmonary vasomotor nerve responses, (4) the effect of reducing the partial pressure of oxygen (pO_2) in the blood perfusate on the responses to pulmonary vasomotor nerve stimulation, and (5) the minimal bronchial arterial pressure required to maintain pulmonary vasomotor nerve responses.

METHODS

Mongrel dogs varying in weight from 14 to 29 kg were exsanguinated under local anaesthesia through a cannula inserted into the femoral artery, after premedication with morphine hydrochloride (2 mg/kg subcutaneously). Atropine sulphate (1 mg) and heparin (Boots

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powdered heparin 3 mg/kg dissolved in distilled water) were injected intravenously some minutes before exsanguination. The blood was collected in a glass cylinder containing an amount of heparin approximately equal to twice that injected intravenously, the total dose of heparin used being 9 mg/kg body weight. Penicillin (Crystapen, crystalline sodium Penicillin G, Glaxo, 500 i.u./100 ml. blood) was added to the blood, which was then filtered through one layer of linen (mesh 50 μ by 250 μ) resting on a stainless-steel mesh (250 μ by 250 μ). The blood was stored at 36° C until used for perfusing the lungs.

In some experiments the left lung was perfused through both the pulmonary and bronchial circulations by the method described by Daly (1956). In others, either the left apical and cardiac lobes or the left lower lobe were perfused by a similar method but with a reduced volume of the blood reservoir (250 ml.) (Daly & Waaler, 1960).

All glass pieces were cleaned and brushed in tap water, soaked in chromosulphuric acid for 24 hr, rinsed ten times in running tap water and twice with distilled water. The rubber connexions were soaked in running tap water for several hours, boiled in 2 % $NaHCO_3$, rinsed in running tap water and finally in distilled water.

The pulmonary circulation was perfused at a constant-volume blood inflow or at a constant head of pressure at 14–22 mm Hg, and the bronchial circulation, including the vascular territory supplying the nerves to be stimulated, at a mean pressure of 80–140 mm Hg. The pulmonary arterial pressure was recorded with a Marey tambour and the left atrial blood outflow with a modified Gaddum blood-flow recorder. The left atrial pressure was recorded by a blood manometer, the top of which was connected to a piston recorder, or taken as the difference in levels between the mouth of the left atrial cannula and the blood as it spilt over into the flow recorder. This latter measurement was found by experiment to be a few millimetres of blood lower than the true left atrial pressure at the blood flow used.

The pulmonary vascular resistance (PVR) was calculated according to the formula

$$\text{PVR} = \frac{\text{Pulmonary arterial pressure (mm Hg)} - \text{Left atrial pressure (mm Hg)}}{\text{Left atrial flow (l./min)} \times x/\text{m}^2},$$

where x equals the ratio of total lung weight to the weight of the perfused lobes, as given by Rahn & Ross (1957). When this formula is used, the PVR for normal resting dogs ranges from 1·7 to 7·5.

Lung ventilation. The lung lobes were ventilated by starting a Starling 'Ideal' pump (C. F. Palmer Ltd.) at a peak pressure of 80–100 mm H_2O with pure O_2, 6 % CO_2 in O_2 or 6 % CO_2 in air. Hypoxaemia was produced in seven tests by changing the ventilation from 6 % CO_2 in O_2 to 6 % CO_2 in N_2, and in one test by changing the ventilation from O_2 to N_2. The tidal air was measured by the method of Konzett & Rössler (1940).

Tests for pulmonary vasomotor activity. The upper thoracic sympathetic chain, the stellate ganglion, the ventral branch of the ansa subclavia or the left thoracic vagosympathetic nerve was stimulated by square-wave impulses derived from an Attree (1950) stimulator. The predominant response to stimulation of the sympathetic fibres in this nerve pathway is pulmonary vasoconstriction (Daly, Duke, Hebb & Weatherall, 1948).

Atropine sulphate equivalent to about 0·05 mg/kg body weight was injected intravenously before exsanguination, or added to the blood reservoir during perfusion, in order to suppress any passive pulmonary circulatory effects due to 'bronchoconstrictor' responses during stimulation of the left thoracic vagosympathetic nerve.

Blood analysis. Blood samples were taken from the pulmonary artery inflow tubing during the course of each experiment. The temperature of the blood perfusate at the time of sampling, usually about 34–35° C, was registered. The samples were stored under paraffin oil at 4° C until the pH measurement and blood gas analysis could be carried out.

The oxygen capacity, the degree of oxygen saturation and the carbon dioxide content of the blood were determined with a modified Haldane blood gas apparatus (Shaw, 1959) by the method of Courtice & Douglas (1947).

Blood pH was measured at room temperature with a Pye 'Master' pH meter (Cat. No. 11068, W. G. Pye and Co.). The apparatus was standardized before each determination by a buffer of pH 7·01 at 20° C. Duplicated estimations agreed within 0·02 pH units or less. The values of blood pH resulting from these measurements at room temperature were converted to pH values corresponding to the blood temperatures in the preparations at the time of sampling by using the temperature coefficient of Rosenthal (1948). Some pH measurements were carried out at 35° C with a 'Vibron' 33B electrometer (Electronic Instruments Ltd.) in conjunction with a 'Vibron' C 33B pH measuring unit. The accuracy of these measurements was at least as great as those done with the 'Master' pH-meter.

Blood pO_2 values were calculated from the oxygen saturation values using the nomogram and the correction factors (for pH and temperature) given by Severinghaus (1958) on p. 73 in *Handbook of Respiration*, assuming that the oxyhaemoglobin dissociation curve for dog follows that of man. Maximal errors in our calculated pO_2 values, arising from errors in the measurements needed (oxygen saturation, blood pH and blood temperature), could be calculated to be of the order of ± 1 mm Hg and ± 3 mm Hg in the pO_2 regions of 25 and 50 mm Hg respectively.

pCO_2 values were calculated from the values for CO_2 content, pH, oxygen capacity and percentage oxygen saturation, by the formula:

$$pCO_2 = \frac{CO_2}{S(10^{(pH-pK')}+1)}$$

(Severinghaus, Stupfel & Bradley, 1956*a*). The plasma CO_2 content (mM/l.) was calculated by multiplying the value for whole blood by the factor '*f*' read from the nomogram of Van Slyke & Sendroy (1928). The solubility factor, *S*, was read from the table of Severinghaus *et al.* (1956*a*), and the value for pK′ from the nomogram of Severinghaus, Stupfel & Bradley (1956*b*).

RESULTS

All the procedures necessary for the separation from the thorax of the reduced systemic circulation, together with the heart and lung lobes to be perfused, were carried out after exsanguination. The period of time which elapsed between exsanguination and the moment when pulmonary circulation perfusion, ventilation and bronchial circulation perfusion (in that order) were established, varied from 45 to 314 min (mean: 132 min) in 52 experiments.

Survival of pulmonary vasomotor nerves during bronchial circulation ischaemia. In most experiments perfusion of the pulmonary circulation and lung ventilation were started before perfusion of the bronchial circulation. Stimulation of the sympathetic nerve path or of the associated ganglia failed to produce a pulmonary vasopressor response before perfusion of the bronchial circulation. Within 2–3 min of the start of bronchial circulation perfusion small but definite pulmonary vasopressor responses to nerve stimulation were obtained. For any given strength of stimulus these reached a maximum after 22–35 min of perfusion. A more accurate assessment of the duration of bronchial circulation perfusion required to establish maximal responses to nerve stimulations could not be obtained because the stimuli were applied at intervals of 10 min. This interval of time was selected because it had been found by experience that it ensured

consistent pulmonary vasomotor responses to successive stimuli over a period of some hours. The records of Fig. 1, which are typical of a large number of experiments, show the absence of a pulmonary vasopressor nerve response before bronchial circulation perfusion (12.01 p.m.), a small response 2 min after the start of bronchial circulation perfusion (12.11 p.m.) and a maximal response 22 min after the start of perfusion (12.31 p.m.).

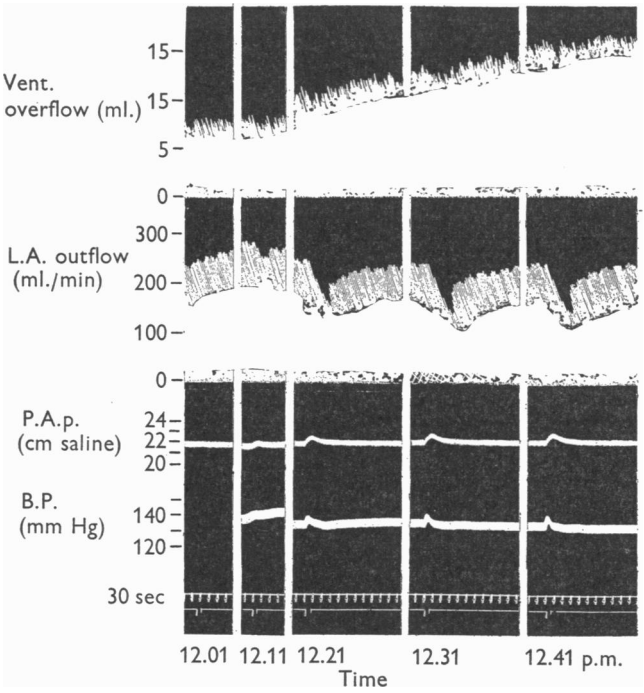

Fig. 1. Effect of stimulating sympathetic post-ganglionic nerve fibres (in left thoracic vagosympathetic nerve) on an atropinized perfused lung lobar preparation before and during additional perfusion of bronchial circulation. Dog: *f*, 17·0 kg, 0·75 m² body surface; perfusion of left apical and cardiac lobes at a constant head of pressure. Bronchial circulation perfusion started at 12.09 p.m. Stimulations during 15 sec periods, 10 V, square-wave impulses of 10 m-sec duration and frequency 47/sec.

Calculated total pulmonary vascular resistance (PVR) values before stimulations and at peak of vasoconstrictor responses: 12.01 p.m., 6·3–6·3; 12.11 p.m., 5·6–6·4; 12.21 p.m., 6·5–10·6; 12.31 p.m., 6·8–12·1; 12.41 p.m., 6·7–11·7. Vent. = ventilation. L.A. = left atrial. P.A.p. = pulmonary arterial pressure. B.P. = perfusion pressure of aorta and bronchial arteries.

These pulmonary vasopressor responses once established were obtainable during short periods of interruption of bronchial circulation perfusion (see Figs. 2 and 3), thus demonstrating that they were not caused by passive bronchial vascular effects on the pulmonary circulation. Since the vaso-

pressor responses occurred in the absence of changes in lung hindrance they were due to pulmonary vasoconstriction.

The experiment in Fig. 1 was carried out at constant head of perfusion pressure, and similar results were obtained in other preparations perfused at constant blood volume inflow.

In spite of the prolonged period of initial bronchial circulatory ischaemia, which in one experiment was deliberately extended to 314 min, well marked pulmonary vasoconstrictor responses to nerve stimulation were always obtained within 35 min of the start of bronchial circulation perfusion.

Extinction of pulmonary vasomotor nerve responses by interruption of bronchial circulation perfusion

Interruption of bronchial circulation perfusion caused a gradual decrease and, finally, extinction of the responses to sympathetic nerve stimulation within 9–38 min. On restarting bronchial circulation perfusion the responses recovered within from 7 to 40–50 min. The reduction in the response occurred within a few minutes of bronchial circulation interruption. This is shown in Fig. 2 in two experiments in which the responses were

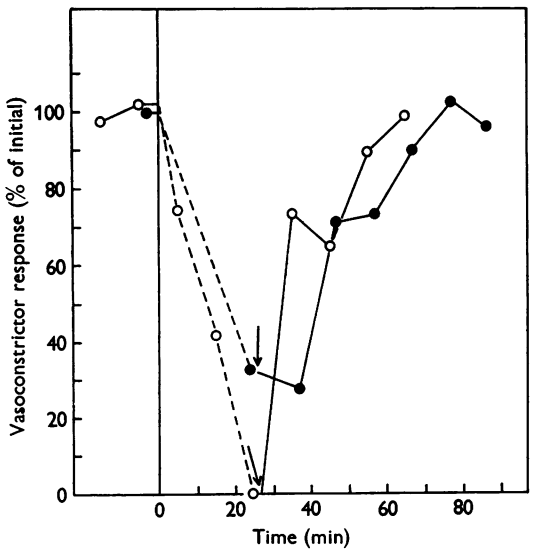

Fig. 2. Effect of interruption of bronchial-circulation perfusion on pulmonary vasoconstrictor responses to stimulation of post-ganglionic sympathetic nerve fibres (in left thoracic vagosympathetic nerve) in two isolated perfused left lung lobar preparations of the dog. Responses expressed as % of initial values. Each point represents the response to one stimulation. Bronchial circulation perfusion interrupted at zero time, restarted at the arrows; the broken lines thus indicating periods of no bronchial circulation perfusion.

due to stimulation of the post-ganglionic fibres in the left thoracic vago-sympathetic nerve. In one experiment (open circles) the stimuli were applied at 10-min intervals and extinction of the response occurred 24 min after interruption of bronchial circulation perfusion; full recovery of the response was obtained 38 min after restarting perfusion. In the other experiment shown stimuli were withheld until bronchial circulation

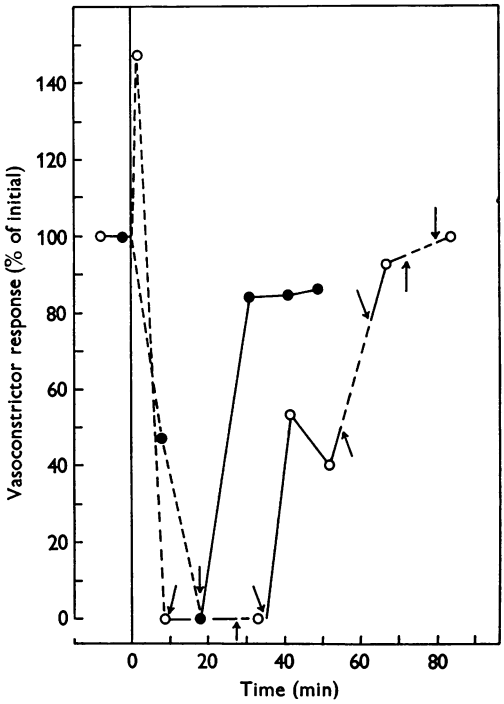

Fig. 3. Effect of interruption of bronchial circulation perfusion on pulmonary vasoconstrictor responses to stimulation of preganglionic sympathetic nerve fibres or of a sympathetic ganglion. Isolated perfused left lung preparations of the dog. ○ an experiment in which the left sympathetic chain was stimulated; ● an experiment in which the left stellate ganglion was stimulated. Responses expressed as % of the initial values. Each point represents the response to one stimulation. Bronchial circulation perfusion interrupted at zero time and at all arrows pointing upwards, perfusion restarted again at all arrows pointing downwards, the broken lines thus indicating periods of no bronchial circulation perfusion.

ischaemia had been established for 23 min, after which the response obtained was 33 % of its initial value. Restarting perfusion of the bronchial circulation produced, after a preliminary fall, full recovery of the response within 40–50 min. Similar results were obtained in three additional experiments of the same type.

The extinction of the responses during bronchial circulation interruption

to stimulation of the upper thoracic sympathetic chain and of the stellate ganglion was tested in three experiments of which two are shown in Fig. 3. It will be seen that the responses were abolished in 9 and 18 min, respectively, rather earlier than the extinction of the responses to stimulation of the post-ganglionic fibres in the thoracic vagosympathetic nerve (Fig. 2). Another feature of these experiments was the appearance of a supernormal response to the first stimulus following bronchial circulation interruption (Fig. 3). Such an initial supernormal response was observed in two out of five bronchial circulation interruption tests in which the sympathetic chain was stimulated and in one out of four tests in which the thoracic vagosympathetic nerve was stimulated.

Hypoxaemia and pulmonary vasomotor nerve responses. In the perfusion system used the two pumps perfusing the pulmonary and bronchial circulations—including the extrapulmonary nerves—drew their blood from a single reservoir. After passage through the lungs the blood from the left atrium flowed through the blood outflow recorder to be collected in the reservoir, and this part of the blood circuit was open to the atmosphere. It was found that during N_2 ventilation the pO_2 of the blood from the left atrium was lower than that of the blood in the reservoir. This was because the blood picked up some oxygen during its passage through the flow recorder and whilst it spilt over into the reservoir. For this reason the blood perfusing both circulations did not reach the low pO_2 values which might be expected if a closed blood-circuit system had been used. Thus, in eight tests in which the lung lobes were ventilated with N_2 for periods of 23–76 min the pO_2 of the blood only reached 26–38 mm Hg (mean 32 mm Hg). It should be emphasized that under these conditions of perfusion N_2 ventilation reduced the pO_2 of the blood circulating through all the tissues of the lungs and also that supplying the extrapulmonary nerves. In seven out of the eight tests mentioned above N_2 ventilation reduced the pulmonary vasoconstrictor responses to stimulation of the sympathetic post-ganglionic fibres in the left thoracic vagosympathetic nerve. In the remaining experiment the responses were not altered. Figure 4 illustrates the results of two of these tests. In one, N_2 ventilation for 30 min produced a moderate reduction in the vasoconstrictor response to nerve stimulation and complete recovery followed ventilation with 6% CO_2 in O_2. In the other the reduction in the response was much greater and recovery was incomplete.

There was no correlation between the reduction in pulmonary vascular responses to nerve stimulation during N_2 ventilation and the relatively small changes in pH and blood pCO_2 which accompanied the fall in blood pO_2.

The two tests in Fig. 4 are given as examples because N_2 ventilation

itself produced no appreciable changes in PVR. In other experiments, however, this type of preparation responded to N_2 ventilation by a fall in PVR. Such a *fall* occurred in five out of the eight tests described above, and in these five experiments also the vasomotor responses to nerve stimulation were reduced, the percentage reduction falling between those shown in Fig. 4. It is realized that the pulmonary vascular depressor effect of hypoxia, which occurred in these experiments, is contrary to the

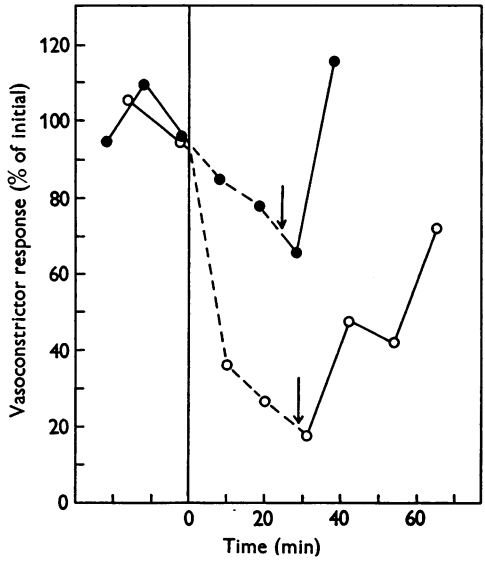

Fig. 4. Effect of temporary N_2 ventilation on pulmonary vasoconstrictor responses to sympathetic nerve fibre stimulation in two isolated perfused left lung lobar preparations of the dog. Post-ganglionic fibres of left thoracic vagosympathetic nerve stimulated. Responses expressed as % of the initial values. Each point represents the response to one stimulation. At zero time ventilation changed from 6 % CO_2 in O_2 to 6 % CO_2 in N_2. Ventilation changed back to 6 % CO_2 in O_2 at arrows, the broken lines thus representing periods of N_2 ventilation.

findings of the majority of other investigators. This is all the more surprising since a vasopressor response to N_2 ventilation was never observed in our experiments. Our perfusion technique differed from that of others who have investigated the effect of hypoxia on the circulation in isolated lung preparations, in that the initial period of pulmonary circulation ischaemia was relatively long and that additional perfusion of the bronchial circulation was carried out. To what extent these factors have determined the vasomotor response to N_2 ventilation is being explored, and will not be further discussed here since it is not obviously relevant to our present examination of the effect of N_2 ventilation on pulmonary vasomotor nerve

responses. These responses were reduced during N_2 ventilation irrespective of the direct effect of N_2 ventilation on the pulmonary circulation.

In one experiment the addition of KCN to the perfusate in the reservoir, to give a blood concentration of 0·1 mg/ml., abolished the pulmonary vasomotor nerve responses within 25 min.

Systemic arterial pressure and pulmonary vasomotor nerve responses

Three experiments were performed to determine the minimal arterial pressure in the bronchial circulation and in the vessels supplying the extra-pulmonary nerves required to maintain normal pulmonary vasomotor nerve response. The post-ganglionic fibres in the left thoracic vago-sympathetic nerve were stimulated for this purpose. It was found during

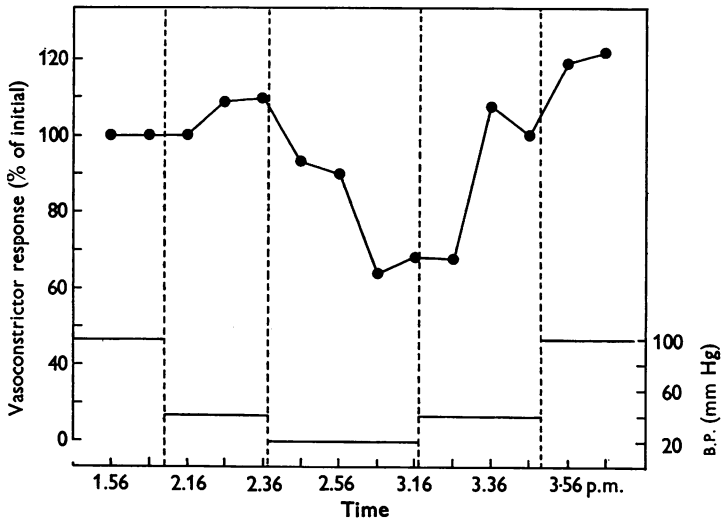

Fig. 5. Effect of altering the perfusion pressure (B.P.) in the bronchial arteries (and aorta) on pulmonary vasoconstrictor responses to sympathetic nerve fibre stimulation (post-ganglionic fibres of left thoracic vagosympathetic nerve) in an isolated perfused left lung lobar preparation of the dog. The points in the upper tracing represent responses to stimulations at 10 min intervals. Responses expressed as % of initial values. The lower lines indicate bronchial circulation perfusion pressure.

two separate tests that a perfusion pressure of 40 mm Hg sufficed to maintain normal responses, but in each test reducing the pressure below this value led to a reduction in the pulmonary vasomotor nerve responses. One of these experiments is illustrated in Fig. 5. A third test on lowered bronchial circulation perfusion pressure showed reduced vasomotor nerve responses when the pressure was brought down to 70 mm Hg or less.

DISCUSSION

The results confirm that in isolated perfused lungs of the dog perfusion of the bronchial circulation is essential for the maintenance of pulmonary vasoconstrictor responses to sympathetic nerve stimulation. The observations that an aortic (bronchial arterial) pressure as low as 30–40 mm Hg or intermittent interruption of bronchial circulation perfusion for 10 min alternating with 10-min periods of perfusion (Fig. 3) can suffice to maintain the responses to their full value indicates that only a relatively small blood flow in the bronchial circulation is necessary to maintain functional activity of the nerves. This is in agreement with the findings of Eccles (1935) and Bargeton (1938) in studies on the superior cervical ganglion.

It seems reasonable to conclude from the experiments with lowered oxygen tension that one of the functions of the bronchial circulation is the maintenance of an oxygen supply to the pulmonary nerves and/or the neuromuscular apparatus. We have little evidence as to the possible role of other blood components supplied by, or removed by, the bronchial circulation. It was, however, observed that addition to the blood reservoir of 'new' blood (i.e. blood which has been stored in a thermostat at 36° C since the death of the animal) sometimes caused a full or a partial restoration of vasomotor responses to nerve stimulations in preparations in which such responses had started to decline. The oxygenation of the perfusate was adequate throughout these experiments, so that other blood components than oxygen must have been responsible for the effect. We have at present no indication as to the nature of this potentiating effect of 'new' blood.

In two experiments it was observed that a stimulus applied to the sympathetic chain or the stellate ganglion just after the bronchial circulation perfusion had been interrupted caused a larger pulmonary vasoconstrictor response than the control value (Fig. 3). The number of experiments is too small to assess the significance of this observation, which, nevertheless, recalls that Bronk & Larrabee (1937) observed an increase of the electrical response and a persistent facilitation in a ganglion during the initial period after it was deprived of its blood supply.

It should be emphasized that the method of perfusion used differed in two respects from that generally practised in setting up isolated perfused lungs. First, there was a prolonged period of pulmonary circulation ischaemia before the start of its perfusion, and secondly all the tests were made during bronchial circulation perfusion, or after a short interruption of such perfusion. How far the initial period of ischaemia affected the pulmonary vascular responses to sympathetic nerve stimulation is uncertain, because under the conditions of the experiment the pulmonary

vascular response before the period of circulatory ischaemia could not be assessed. The experiments show, however, that some of the sympathetic vasoconstrictor fibres are strongly resistant to ischaemia of the bronchial vascular system, in that after prolonged periods of such ischaemia their functional activity can be gradually restored by starting up bronchial circulation perfusion. That the revived nerve fibres in most of the experiments probably represent a high proportion of all those present in the nerve trunk stimulated is suggested by the responses being no smaller than those obtained by Daly *et al.* (1948) in the perfused living animal preparation, in which bronchial circulation ischaemia was absent and there was only a short period of pulmonary circulation ischaemia whilst the preparation was being made.

A puzzling feature of the present series of experiments has been the absence of sympathetic vasodilator responses to nerve stimulations. Such responses, although relatively rare, can be evoked in the perfused living animal (Daly *et al.* 1948; Daly & Daly, 1959). The explanation may be that these vasodilator fibres are less resistant to bronchial circulation ischaemia than the sympathetic pulmonary vasoconstrictor fibres. Since all the perfused preparations were atropinized it was not feasible to examine the effects of ischaemia or of ventilation hypoxia on the atropine-sensitive pulmonary vasodilator fibres described by Daly & Hebb (1952).

The fact that the pulmonary vascular resistance was in the majority of experiments within normal limits for periods of perfusion of 4 hr, during which consistent pulmonary vasoconstrictor nerve responses were obtained, indicates a stability of the pulmonary vascular bed the greater part of which was being perfused. These consistent responses were obtained in experiments in which the lung hindrance gradually increased, thus suggesting that interstitial oedema was not an important factor in determining the responses.

The relation between the rate of decay of pulmonary vasoconstrictor responses and the duration of interruption of the bronchial circulation is of particular interest, since in testing for pulmonary vasomotor nerve activity it is essential to carry out some of the nerve stimulations during temporary interruption of bronchial circulation. Persistence of the pulmonary vasomotor responses during such interruptions excludes the possibility that they are due to passive effects on the pulmonary vascular bed caused by vasomotor changes in the bronchial vascular system. Temporary bronchial circulation interruptions usually lasted for 1–3 min only, and the observations reported indicate that during such periods the vasoconstrictor response to stimulation of post-ganglionic sympathetic fibres can be expected to be reduced by 15% or less.

SUMMARY

1. In atropinized perfused left lung lobe preparations of the dog, with additional perfusion of the bronchial circulation and of the vascular territories supplying the thoracic pulmonary nerves, stimulation of the nerve pathway carrying pulmonary sympathetic fibres (sympathetic chain, stellate or middle cervical ganglion and thoracic vagosympathetic nerve) produced vasoconstrictor responses.

2. The constrictor response was extinguished in 10–35 min by interruption of bronchial circulation perfusion and recovered in about the same period on restarting this perfusion.

3. Ventilation with 6 % CO_2 in N_2, which reduced the pO_2 of the perfusate to 26–38 mm Hg, diminished but did not extinguish the vasoconstrictor response to sympathetic nerve stimulation. This diminution in response could not be correlated with the small changes in pCO_2 and pH which accompanied the changes in pO_2.

4. Bronchial arterial pressures below 40 mm Hg caused a diminution in the pulmonary vasoconstrictor response to sympathetic nerve stimulation in two out of three experiments.

5. Some of the pulmonary vasoconstrictor nerve fibres survive periods of bronchial circulation ischaemia lasting 5 hr.

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