Two recent independent experimental studies have found evidence that ventilation may be modified by changes in the pulmonary vascular bed. Firstly, small amounts of lobeline injected intravenously initiate reflex depression of ventilation mainly from the extrapulmonary parts of the pulmonary arteries of the cat. Larger doses elicit, in addition, cardiovascular changes, bradycardia, and hypotension. Secondly, a fairly rapid reduction of pressure in the pulmonary vascular bed produces a reflex increase in rate and depth of ventilation in the dog. This reflex appears to be tonically active in the normally ventilating animal. A subsequent collaborative study showed that this reflex ventilatory response also occurred in the cat and demonstrated that the afferent pathways for these ventilatory changes have many common features. It was found that a variety of experimental changes (viz. vagal cooling, procaine infusion, and the administration of small amounts of pentobarbital) each affected the ventilatory response to either stimulus (viz. a fall in pulmonary artery pressure and lobeline) very similarly, and distinguished them unequivocally from the pathways for the reflex ventilatory responses phenyldiguanide and veratridine. Both these drugs affect other intrathoracic sensory endings to cause reflex changes in ventilation. This evidence suggested tentatively that reflex changes in ventilation could be initiated by changes in pressure probably confined to the extrapulmonary parts of the pulmonary arteries. This region in the dog contains most of the pulmonary artery mechanoreceptors and in a preliminary study the afferent activity of multiferter preparations of the afferent nerves from this region was shown to increase following lobeline injection.

In this study, the afferent responses of single neurone preparations of pulmonary artery mechanoreceptors to lobeline injection and to changes of pulmonary artery pressure were investigated in the anaesthetized cat whilst pulmonary artery pressure was recorded from a catheter in the pulmonary artery. Search was made in the right cervical vagus for a fiber with arterial mechanoreceptor-like activity as the afferent pathway of the lobeline- and pressure-induced ventilatory reflex is predominantly but not exclusively right sided in this animal. A series of preliminary tests was developed which allowed possible pulmonary fibers to be separated from other arterial mechanoreceptors without opening the chest. It was considered important to keep the chest closed because opening the pleural cavity not only modifies the cardiovascular response to lobeline but also reduces the time during which it can be elicited (unpublished results). All pulmonary artery mechanoreceptors, except one, responded and all other fibers studied originating from the heart, lungs, and blood vessels, were insensitive to intravenous doses of lobeline sufficient to cause ventilatory change. These results extend the evidence that the pulmonary lobeline-sensitive reflexogenic zone is a pressure-sensitive area located in the region of the pulmonary artery bifurcation and that the reflex changes in ventilation caused by this drug are due to its action at this site.

**Methods**

Large, healthy cats weighing between 4 and 5 kg were anaesthetized with pentobarbital (35 mg/kg iv). The trachea was cannulated and systemic arterial pressure was recorded from the
femoral artery by a mercury manometer. A polyethylene catheter was passed via a saphenous vein up to the right atrium. The arrival of the tip of the catheter at the right heart could be detected by resistance to its passage. The catheter was then withdrawn approximately one inch and fixed in position by sutures to the saphenous vein. Subsequent postmortem examination showed that the tip of the catheter was situated invariably in the inferior vena cava between the caval opening into the right atrium and the diaphragm.

A pliable double lumen balloon catheter was prepared from polyethylene tubing. The inner tube, which extended 1 mm beyond the balloon was used to record pressure. It was connected to a Statham transducer (P23Gb) and filled with heparinized saline. The output of the transducer which was passed into a Davis carrier pre-amplifier was displayed on one trace of a dual beam Cossor oscilloscope. The outer concentric tube of the catheter led to the air-filled balloon which, when fully inflated, was 5 to 6 mm in diameter. The catheter, with the balloon partly inflated, was passed into the pulmonary artery. The position of the tip was assessed by noting changes in the pulse wave form. After the catheter tip had obviously entered the artery as shown by the pulse pressure, the balloon was deflated and the catheter slowly withdrawn until a ventricular pulse form was recorded. Finally, the catheter was slowly re-admitted into the artery. By such technique, it was possible to place the catheter tip in position within the pulmonary artery, and the balloon either at the pulmonary ring or immediately distal to the valve. When designing the catheter it was difficult to find an inner tube of such diameter that the arterial pressure was not significantly dampened and yet have the catheter sufficiently pliable to allow this blind placement. After a number of trials, an internal diameter of 0.5 mm for the inner tube of the catheter was found most satisfactory.

Single or few-fiber neurone preparations were obtained by repeated subdivision of the peripheral end of the cut right vagus in the neck, using a technique similar to that of Paintal. The nerve was laid on a black perspex platform, the sheath removed and then split; a binocular dissecting microscope was used. Two saline wick electrodes recorded the afferent impulse activity which, amplified by a conventional R-C coupled amplifier, was displayed with the pulmonary artery pressure on the oscilloscope. On some occasions the ECG was substituted for the pressure trace.

The position of the receptor was determined at the conclusion of the experiment by punctate probing whilst the animal was still alive. This reduced the percentage of receptors whose activity was inevitably lost before localization when the animal was killed prior to this procedure. To accomplish this punctate probing the cat was artificially ventilated, and the chest was opened. Through a puncture wound in the right ventricle, a probe was then passed through the pulmonary valve and the pulmonary artery mechanoreceptors were localized by noting the probing position from which the normal activity of the receptor was increased. The distal parts of the right and left arteries could not be explored by this technique, however. Finally, the animal was killed by injection of an overdose of anaesthetic, the artery was separated from the heart at the conus, and the position of the receptor confirmed and/or the smaller pulmonary arteries additionally explored. Even if positive pulmonary localization was thus made, the great vessels and in particular the known sites of systemic mechanoreceptors were also systematically probed.

Electrophysiological results of afferent activity are expressed in two ways, as impulses per heart beat and as maximum impulses per heart beat, i.e., maximum impulses in 0.1 sec interval per heart beat. The film speed was 2.5 inches per second.

Lobeline and other drugs were administered in a volume of saline less than the intraluminal volume of the catheter (0.3 ml) and washed into the circulation with twice this volume.

Results

A. IDENTIFICATION OF PULMONARY ARTERY MECHANORECEPTORS IN THE INTACT CAT

Neurones with mechanoreceptor activity in the right vagus, may arise from various sites, viz. pulmonary artery, brachiocephalic artery, right common carotid artery, and coronary arteries. Hence, it was necessary to select from such afferent vagal fibers as showed an arterial mechanoreceptor rhythm, those that originated from endings in the pulmonary artery, prior to final proof of their origin by punctate localization at the end of the experiment. Fortunately, pulmonary artery mechanoreceptors can be distinguished from systemic mechanoreceptors by the close correlation of the afferent activity of the former, and the poor correlation of the latter, with changes in pulmonary artery pressure. When such changes were needed in experiments, pulmonary artery pressure (PAP) was increased by the rapid intravenous injection of 2 ml dextran, or decreased by slow inflation of the intravascular balloon.

These procedures and observations were
performed on 18 cats in which satisfactory records of right vagal arterial mechanoreceptor activity were obtained. In addition, six aortic mechanoreceptor fibers were studied, one from the left aortic nerve of each of six cats.

The increase of phasic impulse activity in an afferent fiber, later proved to be of pulmonary origin, was exactly synchronous with the rise of pulse pressure and of mean pressure in the pulmonary artery, when 2 ml of dextran were rapidly injected into the right atrium (fig. 1a, right). Systemic arterial mechanoreceptor impulse activity on the other hand was never increased until some three heart beats after the rise in PAP (fig. 1a, left). When reduction of impulse activity and the fall of PAP occasioned by balloon

![Diagrammatic representation of the effect of the right atrial injection of 2 ml dextran on the mean pulmonary artery pressure (PAP) and afferent activity of aortic and pulmonary artery pressoreceptors plotted beat by beat. Both injections were made during a period of apnea secondary to artificial hyperventilation. Afferent activity is represented as impulses per heart beat (imp/beat) and maximum frequency per heart beat (freq/beat).](http://circres.ahajournals.org/)

![Two continuous photographic records showing the effect of slow inflation and deflation of balloon in the proximal part of the pulmonary artery on the pulmonary artery pressure and the afferent activity of pulmonary artery (upper record) and aortic (lower record) mechanoreceptors.](http://circres.ahajournals.org/)
inflation occurred simultaneously, the receptor could always be localized to the pulmonary arteries (fig. 1b).

Some neurones, shown later to be pulmonary artery mechanoreceptors by punctate probing, responded as pulmonary endings to the intravenous injection of saline, yet did not immediately change activity when the PAP was lowered. Their activity stayed the same or even increased immediately after balloon inflation. It was presumed in these instances that the balloon was distal to the receptor site. Withdrawal of the inflated balloon to the pulmonary ring, led to the normal pattern of immediate response to balloon inflation. In one instance when withdrawal of the balloon to the valve was not successful in producing corresponding spike and pressure changes, the receptor was localized just beyond the ring.

During the course of a long experiment the catheter tip was sometimes displaced passively along the artery. For this reason, the effect of balloon inflation on the receptor activity was noted a number of times in the course of an experiment and the catheter was partly withdrawn when necessary in order to correct this situation.

B. RELATIONSHIP BETWEEN AFFERENT IMPULSE ACTIVITY AND PULMONARY ARTERY PRESSURE

Figure 2 shows the relationship between the number of impulses per cardiac cycle and the mean PAP determined during a slow inflation of the intravascular balloon. As the transmural pressure (pulmonary artery pressure minus intrapleural pressure) was not measured, the relationship between impulses per beat and the mean PAP was determined during the apnea that followed a short period of hyperventilation produced by the artificial respiration pump. During apnea the intrapleural pressure in the cat is approximately 4 mm Hg. When the balloon is rapidly deflated, the PAP rises above the control level and the values, obtained during this rise, fall on the same curve as those obtained when the PAP is raised by dextran injection. Consequently the relationship between impulses per beat and mean PAP may be established above and below the resting level (fig. 2).

The curve is a symmetrical “S” shaped curve with a maximum slope in the region of the resting mean PAP, 16.8 mm Hg ± 1.1 (n = 7). The mean number of impulses per cardiac cycle at the position of maximum slope was 7 ± 0.9 (n = 7). When the transformation, impulses per heart beat to probit maximum impulse activity was made, a linear regression of probit afferent activity on mean PAP was found. Thus, the “S” shaped curve has the same shape as an integrated Gaussian distribution curve. Such a relationship is independent of the characteristics of the pulmonary artery wall, as the force-length diagram of this artery at rest and during sympathetic stimulation is nonlinear.15

C. EFFECT OF LOBELINE ON PULMONARY ARTERY MECHANORECEPTORS

Commercially available lobeline is impure and is obtained by extraction from its plant source. Consequently, before use the lobeline used in this study was assayed biologically for its reflexogenic potency. The median
FIGURE 3a
Continuous photographic record of pulmonary artery pressure (upper trace) and the afferent activity of a pulmonary artery mechanoreceptor (large spikes) and right atrial receptor (small spikes) recorded from the right cervical vagus of the cat. Lobeline (45 µg/kg) in 0.5 ml saline was injected at the signal (left side of second strip) during a period of apnea secondary to artificial hyperventilation.

Effective reflex hypotensive dose of the sample, 15 µg/kg, and three times this dose were used routinely. The response of pulmonary artery mechanoreceptors to the intravenous injection of lobeline occurred in two distinct phases, which, for convenience, are referred to as early and delayed.

Early Response
The response to lobeline injected into the thoracic inferior vena cava occurred between one and three seconds, mean 1.3 sec or within two to four heart beats (fig. 3). It reached the plateau of its effect within several seconds. Control volumes (0.3 to 0.5 ml) of saline had no action. The afferent responses to the rapid injection of 2 ml of saline occurred on the average 0.6 sec before that to lobeline. Impulses were increased in number and frequency and the burst of spikes commenced earlier in systole. These changes were not associated with any detectable change in the observed PAP nor with a change in shape of the pulse wave. If pulse wave and pressure changes did occur and were not recorded due to the dampening properties of the catheter, they were not responsible for the change of afferent activity because impulse changes were not seen with control injections of saline. When the effect was minimal or when only very small doses were injected, an increase in peak frequency of impulses was detected, although no change occurred in the absolute number of impulses per cardiac cycle.

An early response similar to or greater than that illustrated in figure 3a,b,c was observed in 10 of the 18 pulmonary mechanoreceptors studied. In seven of the remaining eight, the response was less. It was found that the response of these fibers was demonstrated more convincingly and satisfactorily by injecting lobeline during the apnea which was secondary to artificial hyperventilation and when the control pressure and afferent activity were constant. This is illustrated in figure 3 where the effect of lobeline on the afferent activity of the same pulmonary...
artery mechanoreceptor during apnea secondary to hyperventilation (a, b) is contrasted with that during artificial ventilation (c). Only one proven pulmonary artery mechanoreceptor was completely insensitive to lobeline (45 μg/kg). This was localized to the pulmonary arterial bed near the right hilum. Because the afferent pathway of the lobeline-induced reflex apnea is carried predominantly in the right vagus and because the right vagus was cut invariably, it was possible to elicit such reflex apnea in only a few animals and then only by using large doses of lobeline. In these instances when the afferent activity of a pulmonary artery pressoreceptor and that of a Hering-Breuer fiber were simultaneously recorded, the mean latency between the afferent response to lobeline injection and the inhibition of the Hering-Breuer activity was 1.5 to 2.0 sec. On no occasion was bradycardia or a reduction of pulmonary artery pressure observed during the early phase.

Delayed Response

Approximately 8 to 10 sec after lobeline injection the initial response was followed by a sudden increase in afferent activity often of very great intensity. Peak frequencies up to 300 impulses per second were then commonly observed. This delayed response was seen in all but the lobeline-insensitive neurone previously mentioned. These exceptionally large increases of frequency were associated, during part of their course at
Diagrammatic representation of the effect of lobeline (45 µg/kg) and a control injection of dextran on the mean pulmonary artery pressure (PAP) and the afferent activity of the same pulmonary artery mechanoreceptor shown in figures 3a and 3b, during mild artificial ventilation. These parameters are plotted beat by beat.

Recruitment
During the course of vagus nerve subdivision, it was common to find a bundle of pulmonary artery mechanoreceptor neurones. When the response of such a bundle to lobeline was studied, afferent fibers previously silent became active in addition to the usual increase of activity observed with fibers active during the control period. These fibers were identified by their characteristic mechanoreceptor rhythm and by a spike height different from any recorded before injection.

Residual Effect
The maximum effect of lobeline during the early response was attained within two seconds of the beginning of the response and was maintained for a further six or more seconds without diminution. In addition, if the relationship between impulses per beat and mean PAP is determined before, and compared with that immediately after, the reflex arterial pressure and ventilatory changes to lobeline have subsided, the curve is shifted to the left. It will be argued in the discussion that both these phenomena suggest a residual effect of the drug.

D. LOCALIZATION OF PULMONARY ARTERY MECHANORECEPTORS IN THE CAT

The position of mechanoreceptors from the right vagus in the pulmonary artery, when localized by the method of punctate probing (fig. 4a), is shown in figure 4b. Although mechanoreceptors were found in all parts of the extrapulmonary parts of the pulmonary arteries, they are most concentrated in the region of the bifurcation.
Photographic record of pulmonary artery pressure (upper trace) and afferent nerve activity to illustrate the procedure of punctate localization. Records from above downwards. A. Activity of presumed pulmonary artery mechanoreceptor (large spikes) and right atrial stretch receptor prior to thoracotomy. B. Increased activity of the pulmonary artery mechanoreceptor during punctate probing of the region of the pulmonary artery bifurcation through a puncture wound in the right ventricle. C. Increased activity of right atrial receptor obtained by probing the anterior wall of the right atrium near the entrance of the superior vena cava.

Receptor locations. Diagram showing distribution of pulmonary artery mechanoreceptors found in the right vagus of cats, and localized by punctate probing of the wall of the pulmonary artery.

E. EFFECT OF VERATRIDINE AND PHENYLDIGUANIDE ON PULMONARY ARTERY MECHANORECEPTORS IN THE CAT

Veratridine (10 μg/kg) injected under the same conditions as lobeline into the thoracic inferior vena cava did not affect the afferent discharge of pulmonary artery mechanoreceptors within 15 seconds of injection. This dose of veratridine causes a reflex cardiovascular change similar to that provoked by 45 μg/kg lobeline. Such injections increased the afferent activity of Hering-Breuer and atrial fibers when these were recorded simultaneously with the mechanoreceptors.

Phenyldiguanide was injected in equipotent reflex hypotensive dose as lobeline, namely 12 and 40 μg/kg. The smaller dose had no action on the afferent discharge of pulmonary artery mechanoreceptors, although the larger dose had a slight effect which appeared after the same latency as the delayed response to lobeline. Such responses were of a trivial nature.

F. EFFECT OF LOBELINE ON OTHER INTRATHORACIC AFFERENT ENDINGS

The effect of lobeline injected into the inferior vena cava in doses up to 45 μg/kg was studied on 12 atrial, 5 ventricular, 12 Hering-Breuer and 12 systemic mechanoreceptors. All these endings were insensitive to this drug injected under these conditions.

Discussion

Lobeline stimulates pulmonary artery mechanoreceptors in intravenous doses that have
no action on other stretch receptors that were studied and which originate in the heart, lungs, and systemic arteries. However, the vagus contains 24 thousand fibers (Paintal, 1963) mostly unmyelinated and it is impossible to say how specific this sensitivity to lobeline may be. Pulmonary artery mechanoreceptors which responded to these doses of lobeline were insensitive to reflexly equivalent doses of veratridine and relatively insensitive to phenylbiguanide. This confirms earlier studies which suggested that the lobeline-sensitive group of neurones is different from those affected by these two drugs. 

The concept that a stretch receptor in addition to chemoreceptors may be sensitive to the nicotine class of drugs, of which lobeline is a member, is not unique. Diamond demonstrated the sensitivity of carotid baroreceptors to nicotine and acetylcholine. These drugs have the same reflexogenic actions as lobeline at the pulmonary artery bifurcation. These findings parallel those of Jarisch et al. who found that veratrum causes reflex changes in doses which fail to excite carotid sinus baroreceptors. It has recently been shown by Coleridge et al. that pulmonary artery mechanoreceptors in the dog are sensitive to capsaicin.

When injected through the same catheter, saline reaches the pulmonary artery as indicated by a rise of pulmonary artery pressure approximately 0.6 sec before the increased afferent response to lobeline is first recorded. This is 0.4 sec less than the shortest latency measured between the injection of lobeline into the region of the pulmonary artery bifurcation and the earliest reflex change. It follows that within 0.4 sec, lobeline must reach the receptor ending from the lumen of the artery and the complex series of changes that eventually result in reflex change must take place. It has been suggested that in the adult a single small arterial branch of the pulmonary artery supplies adventitial structures around the artery. If such an artery is functionally significant, it would distribute lobeline to the widely scattered pulmonary artery mechanoreceptors to cause the early response to the drug and a reflex change of ventilation within these time limits. Because sensory endings have been stained just beneath the pulmonary intima, as well as at all depths of the arterial wall, it is reasonable to conclude that lobeline diffuses to the pulmonary artery mechanoreceptors. Patel et al. have claimed that pulmonary artery flow is zero and sometimes negative during approximately half of each cardiac cycle. If this be so, then it would facilitate the diffusion of even small volumes of drug before it was washed forward by the blood flow into the lungs. If these considerations are correct, the early response to lobeline is related to its diffusion. The experimental observation of a quantitatively varied and comparatively small early response of fibers which all respond dramatically during the delayed response to the drug is consistent with such a view.

If lobeline does diffuse to the sensory endings to cause the early response, then maintenance of the peak pharmacological effect attained after several heart beats for 3 to 4 times this period may be explained only by a local fixation of the drug in the receptor. Hansson and Schmitterlow, using isotopically labelled nicotine, showed that it is fixed selectively by nervous tissue. Although lobeline has a weaker and shorter action than nicotine, the characteristics of its effect on receptors are probably very similar. Lobeline increases the frequency and number of impulses per heart beat and the burst of impulses starts earlier in systole and continues longer into diastole. It also recruits previously silent fibers. Because these effects are the same as would be expected to occur when the mean pulmonary artery pressure and/or pulse pressure are increased, lobeline appears to act by lowering the receptor threshold to its normal stimulus. The actions of drugs on autonomic ganglia and sensory endings have many basic similarities. The findings by Paton and Perry that nicotine acts on ganglia by depolarization suggest that these groups of drugs sensitize sensory endings by a persistent partial depolarization.
When suitable doses of lobeline and other nicotinic drugs were studied, bradycardia occurred in two phases. These phases corresponded in timing with the early and delayed afferent responses of the receptors to these drugs. It seems reasonable that whereas the early phase of the afferent response is caused by local diffusion of lobeline from the lumen of the pulmonary artery, the delayed phase must be due to the action of the drug carried to the receptors via their blood supply, the pulmonary artery vasa vasorum. These originate from the coronary and other systemic arteries.

Assuming that the selection of fibers studied in the right vagus is typical of the total population, the distribution of pulmonary artery mechanoreceptors in the cat is similar to that in the dog, with the exception that, in the cat, the greatest concentration of endings is clearly found at the bifurcation and, that endings are found in the pulmonary artery. This distribution confirms the results of our earlier histologic study of the distribution of afferent nerve endings in the cat.

Summary

1. The effects of lobeline (15 to 45 μg/kg iv) and of local changes of pulmonary artery pressure on the afferent activity of single neurone preparations of pulmonary artery mechanoreceptors have been studied in the intact cat. Pulmonary artery pressure was monitored via a double lumen balloon catheter passed into the pulmonary artery.

2. Pulmonary artery mechanoreceptors were distinguished from systemic mechanoreceptors by applying two simple tests to all mechanoreceptors found by random selection of single fibers from the right cervical vagus. The afferent activity of pulmonary mechanoreceptors closely followed induced changes of pulmonary artery pressure, whereas other arterial mechanoreceptors did not. Pulmonary artery pressure was increased by the rapid injection of 2 ml dextran into the right atrium and was reduced by inflation of the intravascular balloon.

3. In the absence of a rise in pulmonary artery pressure, lobeline increased the afferent activity in two phases. The early response, which was seen in 17 of the 18 fibers studied, occurred within one to two seconds after injection and was characteristically a variable and moderate response. This effect is caused probably by the diffusion of lobeline to sensory endings within the pulmonary artery wall. The delayed response occurred about eight to ten seconds later. The activity of afferent fibers was intensified conspicuously and frequencies up to 300/sec were recorded. It is probable that this is due to the action of lobeline carried by the vasa vasorum to the intramural receptors.

4. By combining the results following balloon inflation and dextran injection, the relationship between impulses per heart beat and mean pulmonary artery pressure was established for a number of pulmonary artery mechanoreceptors in the intact animal.

5. Pulmonary artery mechanoreceptors were insensitive to doses of veratridine and relatively insensitive to doses of phenyldiguanide which, in the intact cat, produce equivalent reflexogenic responses as lobeline (45 μg/kg).

6. Other intrathoracic sensory endings studied, including atrial and ventricular stretch receptors, Hering-Breuer receptors, and systemic mechanoreceptors, were completely insensitive to the doses of lobeline used in these experiments.

7. These results support earlier experiments which suggested that when lobeline is injected, its reflex effect from the pulmonary vascular bed, namely depression of ventilation, is due to its action on a pressure- or stretch-sensitive area in the region of the bifurcation of the pulmonary artery.

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Action of Lobeline on Pulmonary Artery Mechanoreceptors of the Cat

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