Pulmonary Vasodilator Responses to Vagal Stimulation and Acetylcholine in the Cat

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SUMMARY. Responses to vagal stimulation and acetylcholine were investigated in the feline pulmonary vascular bed under conditions of controlled pulmonary blood flow and constant left atrial pressure. Under baseline conditions, electrical stimulation of vagal efferent fibers increases lobar arterial pressure. However, when vasoconstrictor tone was increased, a depressor response was unmasked. The pressor response under baseline conditions and the depressor response under enhanced tone conditions were blocked by phenoxybenzamine and atropine. These data suggest that, in the cat, the vagus is composed of efferent fibers from both the sympathetic and parasympathetic systems. After treatment with 6-hydroxydopamine to destroy the integrity of the sympathetic system, vagal stimulation caused significant frequency-dependent decreases in lobar arterial pressure when lobar vascular tone was increased by infusion of a stable prostaglandin endoperoxide analog or ventilatory hypoxia. Injections of acetylcholine also caused significant dose-related decreases in lobar arterial pressure when lobar vascular resistance was elevated. Depressor responses to vagal stimulation and acetylcholine in 6-hydroxydopamine-treated animals were blocked by atropine and enhanced by physostigmine. Decreases in lobar arterial pressure in response to vagal stimulation in 6-hydroxydopamine-treated animals with enhanced tone were blocked by hexamethonium, whereas responses to injected acetylcholine were not altered by the ganglionic blocking agent. Decreases in lobar arterial pressure in response to vagal stimulation and acetylcholine were similar when the lung was ventilated and when the left lower lobe bronchus was obstructed. In addition, responses to vagal stimulation were similar when systemic arterial pressure was decreased to the level of pressure in the perfused lobar artery. Responses to acetylcholine were not altered after treatment with 5,8,11,14-eicosatetraynoic acid, a lipoxygenase inhibitor. The present data suggest that the feline pulmonary vascular bed is functionally innervated by cholinergic nerves and that vagal stimulation dilates the pulmonary vascular bed by releasing acetylcholine which acts on muscarinic receptors in pulmonary vessels. (Circ Res 53: 86–95, 1983)

THE autonomic innervation of the pulmonary vascular bed has been studied extensively in recent years (Verity and Bevan, 1968; Hebb, 1969; Fillenz, 1970; Kadowitz et al., 1976; Knight et al., 1981). It has been established that the pulmonary vascular bed is innervated by the adrenergic system and that stimulation of the adrenergic nerves increases pulmonary vascular resistance and decreases pulmonary vascular compliance (Ingram et al., 1968; Daly et al., 1970; Kadowitz and Hyman, 1973; Kadowitz et al., 1975). Recent studies in the cat and dog using 5-hydroxydopamine to differentiate adrenergic and cholinergic terminals indicate that the pulmonary vascular bed is innervated by the parasympathetic system (Kadowitz et al., 1976; Knight et al., 1981). Although it has been reported that cholinergic terminals are present in the pulmonary vascular bed, the influence of parasympathetic nerve stimulation is uncertain since, in the study of Daly and Hebb (1952), only small (1–4 mm Hg) inconsistent decreases in pulmonary arterial pressure were observed on a few occasions in response to vagosym-pathetic nerve stimulation in the dog. These authors concluded in their paper that there was no evidence that the pulmonary vasodilator response reflects a change in the caliber of pulmonary vessels and that additional experiments were necessary (Daly and Hebb, 1952). In addition to the uncertainty of responses to vagal stimulation, there is disagreement on responses to acetylcholine in the pulmonary vascular bed. Moreover, both pressor and depressor responses to acetylcholine have been reported, and it has recently been postulated that the vasodilator response to the cholinergic transmitter is due to the release of an endothelial derived product in the lipoxygenase pathway (Furchgott and Zawadski, 1980; Chand and Altura, 1981). The variability in response to acetylcholine may depend on species, experimental preparation, dose of acetylcholine, and on the initial level of tone in the pulmonary vascular bed (Dawes and Mott, 1962; Rudolph and Scarpelli, 1964; Hyman, 1969; Lock et al., 1980). It has also been suggested that the pulmonary vasodilator response to acetylcholine is secondary to its actions.
on the systemic vascular bed (Lock et al., 1980). The present study was undertaken to investigate the actions of vagal stimulation and acetylcholine on the feline pulmonary vascular bed in the intact-chest cat under conditions of controlled pulmonary blood flow and constant left atrial pressure. The results of these studies suggest that neurally released and exogenous acetylcholine act on cholinergic receptors to dilate the pulmonary vascular bed, and that these responses are dependent on the existing level of tone in the bed.

Methods

For studies on the actions of acetylcholine and vagal stimulation on the feline pulmonary vascular bed, 78 adult cats of either sex weighing 2.3-3.7 kg were anesthetized with chloralose/urethane, 50-500 mg/kg, iv, and were strapped in the supine position to a Philip's fluoroscopic table. Supplemental doses of anesthetic were given as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air or room air enriched with 100% O₂. Systemic arterial pressure was measured from a catheter in the femoral artery, and systemic injections of drugs were made through a catheter in the femoral vein.

For perfusion of the left lower lobe, a specially designed 6F triple-lumen catheter was passed under fluoroscopic guidance from an external jugular vein into the arterial branch to that lobe. After the animals had been heparinized, 1000 U/kg, iv, and the lobar artery was isolated by distention of the balloon cuff on the catheter, the lobe was perfused by way of the catheter lumen immediately beyond the balloon cuff. The lobe was perfused with blood withdrawn from femoral artery or vein, and no systematic difference in response to vagal stimulation and acetylcholine was observed when the lobe was perfused with femoral arterial or venous blood. In experiments in which airflow to the left lower lobe was interrupted, the lobe was perfused with arterial blood to lessen the effects of hypoxia. The lobe was perfused by means of a Harvard model 1210 peristaltic pump, and the perfusion rate was adjusted so that lobar arterial perfusion pressure approximated mean pressure in the main pulmonary artery and was thereafter not changed during an experiment. Flow rates to the lobe averaged 46 ± 1 ml/min. These procedures have been described recently (Hyman and Kadowitz, 1979). Left atrial pressure was measured by means of a specially designed 5 or 6F double-lumen catheter placed transseptally into the lobar vein draining the left lower lobe. The catheter tip was positioned in the vein so that the pressure port at the distal lumen was approximately 1 cm into the lobar vein and the second catheter port was at the venoatrial junction. When necessary, blood could be withdrawn or infused through this second catheter lumen to maintain left atrial pressure constant, and in various experiments, left atrial pressure ranged between 2.5 and 4.5 mm Hg. All vascular pressures were measured with Statham transducers zeroed at right atrial level, and mean pressures obtained by electronic averaging were recorded on an Electronics for Medicine recorder model DR-12. In these experiments, a bipolar pacing catheter was positioned in the right ventricle under fluoroscopic guidance, and the bradycardia and/or sinus arrest that occurred during vagal stimulation was eliminated by means of a Medtronic model 5880A demand pacemaker.

In these experiments, responses to vagal stimulation and acetylcholine were investigated when lobar vascular resistance was at resting level and when lobar resistance was elevated by infusions of the prostaglandin endoperoxide analog (15S) hydroxy-11α,9α(epoxymethano)-prosta-5Z,13E dienoic acid or 15-methyl PGE₂ (Upjohn). The endoperoxide analog or 15-methyl PGE₂ was dissolved in 100% ethanol at a concentration of 5 mg/ml, and working solutions were prepared frequently. These substances were infused into the lobar artery with variable speed Harvard infusion pump model 945 at rates which increased lobar arterial pressure by approximately 200% and were 40–180 ng/min for the endoperoxide analog and 120–360 ng/min for 15-methyl PGE₂. The increase in lobar arterial pressure during infusion of the endoperoxide analog or 15-methyl PGE₂ was well maintained, and lobar arterial pressure returned to the control value 5–10 minutes after infusions were terminated.

The adrenergic blocking agents used in these studies were phenoxybenzamine (Dibenzyline; Smith, Kline and French) and propranolol (Ayerst, Sigma). Atropine and hexamethonium (Sigma) were used to block muscarinic and nicotinic (ganglionic) receptors. All blocking agents, with the exception of phenoxybenzamine, were dissolved in 0.9% NaCl solution and were injected slowly over a 2- to 5-minute period into the femoral vein. Phenoxybenzamine was dissolved in a vehicle of ethanol, 10%; propylene glycol, 40%; and 0.9% NaCl, 50% at a concentration of 10 mg/ml and was injected into the femoral vein over a 5-minute period. In experiments in which the effects of the lipoygenase inhibitor, 5,8,11,14-eicosatetraynoic acid (ETYA; Hofman-LaRoche) were investigated, the free acid was reacted with an equivalent amount of NH₄OH, and the resulting clear solution was diluted with 0.9% NaCl to 10 ml and injected iv over a 2- to 5-minute period.

In experiments in which adrenergic neuronal blockade was employed, the animals were treated with 6-hydroxydopamine (Sigma), 100 mg/kg per day, ip, for 3 days and were catheterized on day 4–6. Reserpine (CIBA) was given 1 mg/kg, im, and the animals were catheterized on day 2 or 3.

Agonists used in the study were acetylcholine, norepinephrine, and tyramine (Sigma) and were injected or infused into the lobar artery. For vagal stimulation, the left cervical vagosympathetic nerve was approached through a midline incision on the ventral side in the midcervical region of the neck. The nerve was ligated, and a shielded Falmer electrode was placed around the distal portion of the ligated nerve. The nerve was stimulated with square wave 5 msec duration pulses at supramaximal voltage (5–12 V) for 60–90 seconds with a Grass model SD9 stimulator.

Blood gases and pH were measured with an Instrumentation Laboratory model micro 13 blood gas analyzer. Arterial P0₂, PCO₂, and pH averaged 81 ± 7.44 ± 4 mm Hg, and 7.48 ± 0.04, respectively, in the control period, and were maintained in the physiological range. When necessary, acidosis was corrected by infusion of sodium bicarbonate solution. All hemodynamic data represent peak changes and are expressed in absolute values as mean ± se and perfusion pressures in the various experimental groups are illustrated in Tables 1–6. The data were analyzed by the methods of Snedecor and Cochran (1967) for paired and group comparison. A P value of less than 0.05 was used as the criterion for statistical significance.
Results

Pulmonary Vascular Responses to Vagal Stimulation and Acetylcholine

Pulmonary vascular responses to vagal stimulation and acetylcholine were investigated in the intact chest cat under conditions of controlled pulmonary blood flow. Under baseline (resting tone) conditions in nine cats, stimulation of the left midcervical vagus at stimulus frequencies of 4-16 Hz caused small but statistically significant increases in lobar arterial pressure when left atrial pressure was maintained constant (Fig. 1, top left panel). In three of these animals, the effects of β-receptor blockade on the pressor response to vagal stimulation under baseline conditions was investigated. Vagal stimulation increased lobar arterial pressure 1 ± 0, 2 ± 1, and 3 ± 1 mm Hg at 4, 8, and 16 Hz under baseline conditions, whereas no measurable rise was observed at these stimulus frequencies after administration of phenoxybenzamine, 5 mg/kg, iv. In contrast to the effects of vagal stimulation, intralobar injections of acetylcholine in eight of the cats in doses of 0.5 and 1 μg caused small but statistically significant decreases in lobar arterial pressure under baseline conditions (Fig. 2, top left panel).

Since the magnitude of vasodilator responses in the lung is dependent on the existing level of vasoconstrictor tone which is minimal under baseline conditions [Fio2 = 0.21] (Hyman and Kadowitz, 1979; Hyman et al., 1981), responses to vagal stimulation and acetylcholine were also investigated when lobar vascular tone was elevated. Intralobar infusion of U-46619, a stable prostaglandin endoperoxide analog, n = 9, or 15-methyl PGF2α, n = 3, increased lobar arterial pressure from 12 ± 1 to 36 ± 2 mm Hg in the 12 animals. The increases in lobar arterial pressure in response to the prostaglandin analogs were well maintained during the infusion period. Under enhanced tone conditions in the group of 12 animals, vagal stimulation at 4-16 Hz caused small but statistically significant decreases in lobar arterial pressure (Fig. 1, top right panel). In four of these animals, the effects of β-receptor blockade on the decrease in lobar arterial pressure in response to vagal stimulation were also investigated. Decreases in lobar arterial pressure at stimulus frequencies of 8 and 16 Hz were −2 ± 1 and −4 ± 1 mm Hg before and −2 ± 1 and −4 ± 1 mm Hg after administration of propranolol, 1 mg/kg, iv. However, in eight of the animals, atropine, 1 mg/kg, iv, significantly attenuated the decreases in lobar arterial pressure in response to vagal stimulation at 8 and 16 Hz which were −1 ± 0 and −4 ± 1 mm Hg before and 0 and −2 ± 0 mm Hg after atropine.

Under conditions of enhanced vascular tone, intralobar injections of acetylcholine, 0.05-1 μg, in eight animals caused significant dose-related decreases in lobar arterial pressure (Fig. 2, top right panel). Pressure decreased 19, 27, 45, and 48% at the 0.05-1 μg doses. Responses to acetylcholine were rapid in onset and lobar arterial pressure returned to control values 1-3 minutes after the injection. Lobar arterial pressure decreased −6 ± 1, −8 ± 1, −11 ± 1, and −13 ± 2 mm Hg at the 0.05-1 μg doses of acetylcholine under enhanced tone conditions, and these decreases in pressure were reduced to a similar extent as observed in 6-hydroxydopamine-treated animals after administration of atropine in Table 1.
Figure 2. Dose-response curves for acetylcholine in the intact-chest cat under conditions of controlled pulmonary blood flow and constant left atrial pressure. Upper panels, under resting (control) conditions injections of acetylcholine, 0.5, and 1 µg, caused small but significant decreases in lobar arterial pressure. The decreases in lobar arterial pressure at the 0.5- and 1-µg doses were not enhanced in animals treated with 6-hydroxydopamine (left panel). Under enhanced conditions, intralobar injections of acetylcholine, 0.05-1 µg caused marked frequency-dependent decreases in lobar arterial pressure (right panel). The responses were slow in onset, reaching a steady state 30-60 seconds after onset of stimulation, and the decreases in lobar arterial pressure were well maintained during nerve stimulation for periods up to 90 seconds. Lobar arterial pressure decreased 12, 18, 25, and 38% at frequencies of 2-16 Hz, and pressure returned to control level over a 1- to 3-minute period after vagal stimulation was terminated. When lobar vascular resistance was elevated by infusion of U-46619 (n = 5) or 15-methyl PGF2α (n = 3), intralobar injections of acetylcholine (0.05–1 µg) caused significant dose-related reductions in lobar arterial pressure (Fig. 2, top right panel). These responses were not significantly different from those to vagal stimulation were investigated in animals treated with 6-hydroxydopamine, an agent that interferes with the capacity of adrenergic nerves to store catecholamines (Kostrewa and Jacobowitz, 1974). For these experiments, the animals were treated with 6-hydroxydopamine, 100 mg/kg, ip, for 3 days and were catheterized on day 4-6. In six animals treated with 6-hydroxydopamine, vagal stimulation at 4-16 Hz elicited small but statistically significant decreases in lobar arterial pressure (Fig. 1, top left panel). In these animals, as in control animals, intralobar injections of acetylcholine caused small but significant decreases in lobar arterial pressure at the 0.5- and 1-µg doses (Fig. 2, top left panel). However, when lobar arterial pressure was increased from 13 ± 1 to 34 ± 2 mm Hg in 12 animals by intralobar infusion of U-46619, n = 9, or 15-methyl PGF2α, n = 3, vagal stimulation at 2-16 Hz caused marked frequency-dependent decreases in lobar arterial pressure (Fig. 1, top right panel). The responses were slow in onset, reaching a steady state 30–60 seconds after onset of stimulation, and the decreases in lobar arterial pressure were well maintained during nerve stimulation for periods up to 90 seconds. Lobar arterial pressure decreased 12, 18, 25, and 38% at frequencies of 2-16 Hz, and pressure returned to control level over a 1- to 3-minute period after vagal stimulation was terminated. When lobar vascular resistance was elevated by infusion of U-46619 (n = 5) or 15-methyl PGF2α (n = 3), intralobar injections of acetylcholine (0.05–1 µg) caused significant dose-related reductions in lobar arterial pressure (Fig. 2, top right panel). These responses were not significantly different from those

Table 1

**Effect of Atropine on Responses to Vagal Stimulation and Acetylcholine**

<table>
<thead>
<tr>
<th></th>
<th>Lobar arterial pressure (mm Hg)</th>
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<tbody>
<tr>
<td><strong>Vagal stimulation</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>2 Hz</td>
<td>31 ± 2†</td>
</tr>
<tr>
<td>Control</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>4 Hz</td>
<td>28 ± 1†</td>
</tr>
<tr>
<td>Control</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>8 Hz</td>
<td>25 ± 2†</td>
</tr>
<tr>
<td>Control</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>16 Hz</td>
<td>20 ± 2†</td>
</tr>
<tr>
<td><strong>Acetylcholine</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>0.1 µg</td>
<td>25 ± 1†</td>
</tr>
<tr>
<td>Control</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>0.5 µg</td>
<td>14 ± 2†</td>
</tr>
<tr>
<td>Control</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>1.0 µg</td>
<td>15 ± 1†</td>
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</table>

*When lobar vascular resistance was elevated by U-46619 in 6-hydroxydopamine-treated cats.
† P < 0.05, compared with corresponding control.

Influence of Adrenergic Neuronal Blockade and Hexamethonium

It has been reported that the vagus is a mixed nerve containing efferent fibers from both parasympathetic and sympathetic divisions of the autonomic nervous system (Daly and Hebb, 1952). Therefore, responses to vagal stimulation were investigated in animals treated with 6-hydroxydopamine, an agent that interferes with the capacity of adrenergic nerves to store catecholamines (Kostrewa and Jacobowitz, 1974). For these experiments, the animals were treated with 6-hydroxydopamine, 100 mg/kg, ip, for 3 days and were catheterized on day 4-6. In six animals treated with 6-hydroxydopamine, vagal stimulation at 4-16 Hz elicited small but statistically significant decreases in lobar arterial pressure (Fig. 1, top left panel). In these animals, as in control animals, intralobar injections of acetylcholine caused small but significant decreases in lobar arterial pressure at the 0.5- and 1-µg doses (Fig. 2, top left panel). However, when lobar arterial pressure was increased from 13 ± 1 to 34 ± 2 mm Hg in 12 animals by intralobar infusion of U-46619, n = 9, or 15-methyl PGF2α, n = 3, vagal stimulation at 2-16 Hz caused marked frequency-dependent decreases in lobar arterial pressure (Fig. 1, top right panel). The responses were slow in onset, reaching a steady state 30–60 seconds after onset of stimulation, and the decreases in lobar arterial pressure were well maintained during nerve stimulation for periods up to 90 seconds. Lobar arterial pressure decreased 12, 18, 25, and 38% at frequencies of 2-16 Hz, and pressure returned to control level over a 1- to 3-minute period after vagal stimulation was terminated. When lobar vascular resistance was elevated by infusion of U-46619 (n = 5) or 15-methyl PGF2α (n = 3), intralobar injections of acetylcholine (0.05–1 µg) caused significant dose-related reductions in lobar arterial pressure (Fig. 2, top right panel). These responses were not significantly different from those
The functional extent of depletion of catecholamines from adrenergic nerves was assessed by comparing responses to tyramine, an indirectly acting sympathomimetic agent, in control animals and in animals treated with 6-hydroxydopamine. In control animals (n = 5), intralobar injections of tyramine, 200 μg, increased lobar arterial and systemic arterial pressures 5 ± 1 and 24 ± 4 mm Hg, respectively. In 6-hydroxydopamine-treated animals (n = 4), this dose of tyramine increased lobar arterial and systemic arterial pressure 1 ± 0 and 4 ± 1 mm Hg, respectively. The increases in lobar arterial and systemic arterial pressures were significantly less in 6-hydroxydopamine treated animals, compared with controls. The effect of treatment with 6-hydroxydopamine was further investigated by comparing responses to norepinephrine in control animals (n = 6) and in cats treated with the adrenergic neuronal blocking agent, 100 mg/kg, ip, for 3 days and studied on day 4–6. In control animals, intralobar infusion of norepinephrine, 0.25 μg/kg per min for 2–3 minutes increased lobar arterial pressure from 12 ± 1 to 16 ± 1 mm Hg. In animals treated with 6-hydroxydopamine (n = 4), a similar increase in lobar arterial pressure (10 ± 1 to 13 ± 1 mm Hg) was observed at a norepinephrine infusion rate of 0.025 μg/kg per min which was one-tenth the control rate.

In addition to experiments with 6-hydroxydopamine, the effects of a second adrenergic neuronal blocking agent were investigated in another group of five cats. These animals were treated with reserpine, 1 mg/kg, im, and the animals were catheterized on day 2 or 3. In these animals, vagal stimulation at 8 and 16 Hz and acetylcholine injections at the 1-μg dose caused small but significant decreases in lobar arterial pressure. However, when lobar vascular resistance was increased by infusion of U-46619, vagal stimulation at 4–16 Hz and acetylcholine injections, 0.1–1 μg, caused significant frequency and dose-dependent decreases in lobar arterial pressure (Table 3). Although decreases in lobar arterial pressure in response to intralobar injections of acetylcholine were similar in animals treated with

### Table 2

<table>
<thead>
<tr>
<th>Vagal stimulation</th>
<th>Control</th>
<th>Hexamethonium</th>
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<tbody>
<tr>
<td></td>
<td>38 ± 2</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>4 Hz</td>
<td>33 ± 1 t</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>39 ± 1</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>8 Hz</td>
<td>31 ± 1 t</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39 ± 1</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>0.05 μg</td>
<td>34 ± 1 t</td>
<td>35 ± 1 t</td>
</tr>
<tr>
<td>Control</td>
<td>40 ± 1</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>0.1 μg</td>
<td>31 ± 1 t</td>
<td>32 ± 1 t</td>
</tr>
</tbody>
</table>

* When lobar vascular resistance was elevated by U-46619 in 6-hydroxydopamine-treated cats.
†P < 0.05, compared with corresponding control.

n = 5.
6-hydroxydopamine or reserpine, responses to vagal stimulation were significantly smaller at 4, 8, and 16 Hz in reserpine-treated animals (Table 3; Figs. 1 and 2, top right panel).

Effect of Physostigmine

If responses to vagal stimulation are the result of release of acetylcholine from cholinergic terminals, then these responses should be enhanced by physostigmine, a cholinesterase inhibitor. In another group of cats treated with 6-hydroxydopamine, decreases in lobar arterial pressure in response to vagal stimulation at 1 and 2 Hz, \( n = 5 \), and to acetylcholine at 0.5 and 1 \( \mu \)g, \( n = 5 \), were enhanced significantly after administration of physostigmine, 1 mg/kg, iv (Figs. 1 and 2, left lower panels). Furthermore, when lobar vascular resistance was elevated by infusion of U-46619, the frequency-response curve for vagal stimulation was shifted to the left by physostigmine and the threshold frequency for stimulation decreased from 2 Hz to 0.25 Hz. The dose-response curve for acetylcholine was also shifted to the left by the cholinesterase inhibitor and the threshold dose was reduced (Figs. 1 and 2, right lower panels).

Influence of Systemic Hypotension, Bronchial Obstruction, and ETYA

Although bradycardia and systemic hypotension in response to vagal stimulation were minimized by ventricular pacing, using an electrode catheter in the right ventricle, aortic pressure decreased from 130 ± 5 to 115 ± 6 mm Hg in control animals and from 105 ± 4 to 95 ± 5 mm Hg in 6-hydroxydopamine-treated animals. Moreover, a decrease in systemic arterial pressure could change lobar arterial pressure by altering bronchopulmonary shunt flow (Daly et al., 1948). Therefore, the effects of vagal stimulation were compared when aortic pressure was at normal levels and when pressure was reduced during a period of ventricular fibrillation. In four animals treated with 6-hydroxydopamine, a short period of high frequency stimulation of the right ventricular free wall by way of an electrode catheter induced ventricular fibrillation, during which time, aortic pressure decreased from 110 ± 6 to 50 ± 7 mm Hg. The reduction in aortic pressure had no significant effect on lobar arterial pressure (control 35 ± 2 and 34 ± 2 during fibrillation) or on the response to vagal stimulation at 16 Hz (control 2 Hz 0.25 Hz. The dose-response curve for acetylcholine was also shifted to the left by the cholinesterase inhibitor and the threshold dose was reduced (Figs. 1 and 2, right lower panels).

Responses during Alveolar Hypoxia

The effects of vagal stimulation were also investigated in another group of cats treated with 6-hydroxydopamine when lobar arterial pressure was increased by ventilatory hypoxia. A record from an

### Table 4

<table>
<thead>
<tr>
<th>Lobar arterial pressure (mm Hg)</th>
<th>Vagal stimulation</th>
<th>Acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>4 Hz</td>
<td>27 ± 2†</td>
<td>30 ± 1†</td>
</tr>
<tr>
<td>Control</td>
<td>39 ± 1</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>8 Hz</td>
<td>28 ± 1†</td>
<td>29 ± 1†</td>
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### Table 5

<table>
<thead>
<tr>
<th>Lobar arterial pressure (mm Hg)</th>
<th>Control</th>
<th>Acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36 ± 2</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Acetylcholine (0.1 mg)</td>
<td>30 ± 1†</td>
<td>31 ± 2†</td>
</tr>
<tr>
<td>Control</td>
<td>38 ± 2</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>Acetylcholine (0.5 mg)</td>
<td>30 ± 1†</td>
<td>31 ± 2†</td>
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</table>

\( n = 5 \).

* When lobar vascular resistance was elevated by U-46619.

† \( P < 0.05 \), compared with corresponding control.

It has been reported that 5,8,11,14-eicosatetraynoic acid (ETYA) inhibits the relaxant effect of acetylcholine on isolated vascular smooth muscle, and it has been postulated that release of a vasodilator product in the arachidonic acid lipoygenase pathway may be one of the principal mechanisms for cholinergic vasodilation in vivo (Furchgott and Zawadzki, 1980). To test this hypothesis, the effects of ETYA on decreases in lobar arterial pressure in response to acetylcholine were investigated. In a group of five animals, the decreases in lobar arterial pressure in response to acetylcholine were not altered after administration of ETYA in doses up to 35 mg/kg, iv (Table 5).

\[ \text{Effect of Obstruction of the Left Lower Lobe Bronchus on Responses to Vagal Stimulation and Acetylcholine*} \]

\[ \begin{array}{ccc}
\text{Lobar arterial pressure (mm Hg)} & \text{Control} & \text{Bronchus obstructed} \\
\hline
\text{Vagal stimulation} & 34 ± 2 & 36 ± 2 \\
4 Hz & 27 ± 2† & 30 ± 1† \\
Control & 39 ± 1 & 37 ± 2 \\
8 Hz & 28 ± 1† & 29 ± 1† \\
\text{Acetylcholine} & 36 ± 3 & 35 ± 2 \\
0.05 µg & 28 ± 3† & 30 ± 1† \\
Control & 41 ± 1 & 39 ± 1 \\
0.1 µg & 32 ± 2† & 29 ± 3† \\
\end{array} \]

\( n = 4 \).

* When lobar vascular resistance was elevated by U-46619. 
† \( P < 0.05 \), compared with corresponding control.

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Hypoxia in 6-hydroxydopamine-treated animals, as has been reported previously in the dog (Daly and Hebb, 1952). Since efferent fibers from both sympathetic and parasympathetic systems are represented in the cervical vagus, the effects of 6-hydroxydopamine, an agent which destroys the integrity of adrenergic terminals, on responses to vagal stimulation were investigated (Kostrzewa and Jacobowitz, 1974). Treatment with 6-hydroxydopamine markedly inhibited pressor responses to tyramine and enhanced pressor responses to norepinephrine in the systemic and lobar vascular beds, suggesting that the neuronal blocking agent depleted adrenergic terminals of norepinephrine and inhibited uptake of adrenergic transmitter (Shibata et al., 1972; Kadowitz et al., 1976; Tucker, 1980). After treatment with 6-hydroxydopamine, stimulation of efferent vagal fibers decreased lobar arterial pressure, and responses to vagal stimulation were greatly enhanced when vasoconstrictor tone was increased to a high steady level during infusion of 15-methyl PGF$_{2\alpha}$ or U-46619, a stable endoperoxide analog whose actions may mimic those of thromboxane A$_2$ (Coleman et al., 1981). Since lobar blood flow and left atrial pressure were maintained constant, the reductions in lobar arterial pressure in response to vagal stimulation suggest that pulmonary lobar vascular resistance is decreased. The reductions in lobar arterial pressure in response to vagal stimulation were not modified by propranolol, suggesting that the vasodilator response is not mediated in part through activation of $\beta$-receptors by neuronally released norepinephrine (Hyman et al., 1981). When vasoconstrictor tone was enhanced during infusion of U-46619 or 15-methyl PGF$_{2\alpha}$, intralobar injections of acetylcholine decreased lobar arterial pressure and the response was not altered by treatment with 6-hydroxydopamine. However, decreases in lobar arterial pressure in response to vagal stimulation and acetylcholine in 6-hydroxydopamine-treated animals with enhanced tone were blocked by atropine, a muscarinic receptor-blocking agent. In contrast to experiments with atropine, responses to vagal stimulation and to acetylcholine in 6-hydroxydopamine-treated animals with enhanced tone were greatly increased by phystostigmine, a cholinesterase inhibitor. These data suggest that vagal stimulation decreases lobar arterial pressure by releasing acetylcholine, which acts on muscarinic receptors in the pulmonary vascular bed. Vasodilator responses to vagal stimulation were blocked after treatment with hexamethonium, a ganglionic blocking agent, whereas responses to acetylcholine were not affected after ganglionic blockade. These data indicate that the decreases in lobar arterial pressure in response to vagal stimulation are due to activation of preganglionic cholinergic neurons.

In a recently published study, it has been shown that small and medium size intrapulmonary arteries in the cat have cholinergic terminals (Knight et al., 1981). However, the effects of cholinergic (vagus-sympathetic) nerve stimulation are uncertain, since experiment is shown in Figure 3 and summary data are presented in Table 6. Ventilation with 10% O$_2$ in N$_2$ caused a significant increase in lobar arterial pressure without altering left atrial pressure (Table 6). When lobar arterial pressure was increased by ventilation with 10% O$_2$, vagal stimulation caused a significant reduction in lobar arterial pressure without altering left atrial pressure (Fig. 3; Table 6).

**Discussion**

Results of the present investigation in the intact- chest cat show that, under normal resting conditions, electrical stimulation of the peripheral segment of the vagus nerve in the midcervical region increases lobar arterial pressure. However, when vasoconstrictor tone in the pulmonary vascular bed was increased by several mechanisms, the pressor response was reversed and a depressor response was unmasked. The pressor response under baseline conditions was blocked by phenoxybenzamine, whereas the depressor response under enhanced tone conditions was blocked by atropine. These data suggest that in the cat, in the cervical region, the vagus is composed of efferent fibers from both sympathetic and parasympathetic divisions of the autonomic nervous system, as has been reported previously in the dog (Daly and Hebb, 1952). Since efferent fibers from both sympathetic and parasympathetic systems are represented in the cervical vagus, the effects of 6-hydroxydopamine, an agent which destroys the integrity of adrenergic terminals, on responses to vagal stimulation were investigated (Kostrzewa and Jacobowitz, 1974). Treatment with 6-hydroxydopamine markedly inhibited pressor responses to tyramine and enhanced pressor responses to norepinephrine in the systemic and lobar vascular beds, suggesting that the neuronal blocking agent depleted adrenergic terminals of norepinephrine and inhibited uptake of adrenergic transmitter (Shibata et al., 1972; Kadowitz et al., 1976; Tucker, 1980). After treatment with 6-hydroxydopamine, stimulation of efferent vagal fibers decreased lobar arterial pressure, and responses to vagal stimulation were greatly enhanced when vasoconstrictor tone was increased to a high steady level during infusion of 15-methyl PGF$_{2\alpha}$ or U-46619, a stable endoperoxide analog whose actions may mimic those of thromboxane A$_2$ (Coleman et al., 1981). Since lobar blood flow and left atrial pressure were maintained constant, the reductions in lobar arterial pressure in response to vagal stimulation suggest that pulmonary lobar vascular resistance is decreased. The reductions in lobar arterial pressure in response to vagal stimulation were not modified by propranolol, suggesting that the vasodilator response is not mediated in part through activation of $\beta$-receptors by neuronally released norepinephrine (Hyman et al., 1981). When vasoconstrictor tone was enhanced during infusion of U-46619 or 15-methyl PGF$_{2\alpha}$, intralobar injections of acetylcholine decreased lobar arterial pressure and the response was not altered by treatment with 6-hydroxydopamine. However, decreases in lobar arterial pressure in response to vagal stimulation and acetylcholine in 6-hydroxydopamine-treated animals with enhanced tone were blocked by atropine, a muscarinic receptor-blocking agent. In contrast to experiments with atropine, responses to vagal stimulation and to acetylcholine in 6-hydroxydopamine-treated animals with enhanced tone were greatly increased by phystostigmine, a cholinesterase inhibitor. These data suggest that vagal stimulation decreases lobar arterial pressure by releasing acetylcholine, which acts on muscarinic receptors in the pulmonary vascular bed. Vasodilator responses to vagal stimulation were blocked after treatment with hexamethonium, a ganglionic blocking agent, whereas responses to acetylcholine were not affected after ganglionic blockade. These data indicate that the decreases in lobar arterial pressure in response to vagal stimulation are due to activation of preganglionic cholinergic neurons.

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**Table 6**

<table>
<thead>
<tr>
<th></th>
<th>Lobar artery</th>
<th>Left atrium</th>
<th>Aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13 ± 1</td>
<td>2 ± 0</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>17 ± 1†</td>
<td>2 ± 0</td>
<td>96 ± 4†</td>
</tr>
<tr>
<td>Vagal stimulation</td>
<td>15 ± 1</td>
<td>3 ± 1</td>
<td>87 ± 2†</td>
</tr>
<tr>
<td>(4 Hz)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14 ± 2</td>
<td>3 ± 1</td>
<td>113 ± 7</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>21 ± 1†</td>
<td>3 ± 0</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>Vagal stimulation</td>
<td>16 ± 1</td>
<td>3 ± 1</td>
<td>100 ± 5</td>
</tr>
</tbody>
</table>

$\text{n} = 11$.  
†$P < 0.05$ when compared to control.

**FIGURE 3.** Records from an experiment illustrating the effect of vagal stimulation at 8 Hz on lobar arterial pressure when lobar vascular resistance had been increased by ventilation with 10% O$_2$ in nitrogen. When the Fi$_O^2$ was decreased from 0.21 to 0.10, lobar arterial pressure was increased from 18 to 28 mm Hg in this animal.
reports in the literature show modest increases, modest decreases, a biphasic response, or no change in pulmonary vascular resistance (Daly and Hebb, 1952, 1966). Daly and Hebb (1952) showed in a perfused dog lung preparation that stimulation of the cervical vagosympathetic trunk increased pulmonary arterial pressure in two animals, decreased pulmonary arterial pressure in six, and elicited a biphasic response in two animals. The decreases in pressure in response to vagal stimulation were small (1–4 mm Hg) and were blocked by atropine (Daly and Hebb, 1952). However, these investigators concluded from their results that a final decision as to the existence of atropine-sensitive pulmonary vasodilator fibers must await further studies, including experiments designed to ensure that the pulmonary arterial pressure changes are not secondary to alterations in the transfer of blood from the bronchial (systemic) to the pulmonary circulation (Daly and Hebb, 1952). Results of the present investigation extend the work of Daly and Hebb (1952) by demonstrating that efferent vagal stimulation caused larger-than-previously-recognized, consistent, stimulus-related decreases in lobar vascular resistance in intact-chest animals after treatment with 6-hydroxydopamine when vasoconstrictor tone was increased. The dilator responses were blocked by atropine and enhanced by physostigmine, and similar responses were elicited by intralobar injections of acetylcholine, suggesting that they were cholinergic in nature.

The contribution of the bronchial circulation to the response to vagal stimulation was minimal in these experiments, since transfer of blood from the lobar vascular bed to the systemic vascular bed would not occur when systemic arterial pressure was maintained at normal levels by cardiac pacing. In addition, experiments showing that responses to vagal stimulation and to acetylcholine were similar when systemic arterial pressure was decreased to levels approximately equal to or lower than lobar arterial pressure during a period of ventricular fibrillation suggests that alterations in bronchial blood flow contribute little, if anything, to the lobar vascular response to vagal stimulation or acetylcholine. In addition, a marked reduction in systemic arterial pressure had no significant effect on lobar arterial pressure, suggesting that changes in bronchial flow, which is less than 5% of pulmonary flow, had no measurable effect on lobar hemodynamics in the cat. Similar observations have been made in the intact-chest dog (Hyman et al., 1979; Hyman and Kadowitz, 1979; Hyman et al., 1981; Kadowitz et al., 1981). The physiological significance of the cholinergic dilator system is uncertain at resting tone (FIO2, 0.21) conditions. However, when vasoconstrictor tone is elevated by ventilatory hypoxia (FIO2, 0.10), the present data show that this neurogenic system could produce significant vasodilation. In addition to hypoxic vasoconstriction, pulmonary vascular resistance is increased by prostaglandins and thromboxane A2 in a number of pulmonary disorders, including endotoxin shock and embolism (Casey et al., 1982; Demling et al., 1980; Utsunomiya et al., 1982). The present data suggest that, in pathophysiological states in which tone is elevated by PGE2, or thromboxane A2, the neurogenic cholinergic vasodilator system could produce marked unloading of the right ventricle, since the actions of U-46619 closely mimic those of thromboxane A2 (Coleman et al., 1981).

In all previously discussed experiments in which a vasodilator response to vagal stimulation was described, 6-hydroxydopamine was used to destroy the integrity of adrenergic terminals. To determine whether the vasodilator response to vagal stimulation could be demonstrated when adrenergic neuronal activity is inhibited with another neuronal blocking agent, the effects of reserpine were investigated. Reserpine also impairs adrenergic transmission by depleting nerve terminal stores of norepinephrine (Iggo and Vogt, 1968; Viveros et al., 1969). In animals pretreated with reserpine, vagal stimulation and intralobar acetylcholine injections caused significant decreases in lobar arterial pressure, and these responses were greatly enhanced when lobar
vascular resistance was increased to a high steady level with U-46619. When lobar vascular resistance was increased by the thromboxane analog, U-46619, decreases in lobar arterial pressure in response to vagal stimulation and acetylcholine became frequency- and dose-dependent. However, in reserpine-pretreated animals, decreases in lobar arterial pressure in response to vagal stimulation were significantly smaller than in 6-hydroxydopamine-treated animals when lobar vascular resistance was increased to comparable levels during infusion of U-46619. The explanation for the difference in magnitude of response to vagal stimulation in 6-hydroxydopamine and reserpine-pretreated animals is uncertain. However, responses to acetylcholine were similar in both groups of animals, suggesting that the difference may be related to the extent of adrenergic neuronal blockade achieved with the two agents in these experiments.

Results of the present study suggest that neurally released and exogenously administered acetylcholine decrease pulmonary vascular resistance by acting on muscarinic receptors in the pulmonary vascular bed, and it has been recently reported that acetylcholine relaxes isolated strips of rabbit aorta and canine intrapulmonary artery (Furchgott and Zawadski, 1980; Chad and Altura, 1981). In isolated vessels, relaxation induced by acetylcholine was blocked when endothelium of the vessel was disrupted (Chand and Altura, 1981; Furchgott and Zawadski, 1980), and in one study, responses to acetylcholine were blocked by ETYA, a lipoxygenase inhibitor (Furchgott and Zawadski, 1980). It was therefore postulated that acetylcholine acts on endothelium to stimulate formation of a vasodilator metabolite in the lipoxygenase pathway (Furchgott and Zawadski, 1980), and, based on isolated tissue studies, these authors proposed that this may be one of the principal mechanisms for acetylcholine-induced vasodilation in vivo (Furchgott and Zawadski, 1980). To test the possibility that acetylcholine is dilating the pulmonary vascular bed by releasing a product in the lipoxygenase pathway, the effects of ETYA, a lipoxygenase inhibitor, on responses to acetylcholine were investigated. In these experiments, ETYA in doses up to 35 mg/kg, iv, had no significant effect on the vasodilator response to acetylcholine. These findings are consistent with the results of Chand and Altura (1981) in isolated canine pulmonary arteries and may indicate that a lipoxygenase metabolite does not mediate vasodilator responses to acetylcholine in the pulmonary vascular bed. The difference in effect of ETYA in pulmonary vessels both in vivo and in vitro and in isolated aorta may be due to species variation or to a real difference in the mediation of the response to acetylcholine in the pulmonary circulation and in the aorta, a systemic vessel.

In summary, results of the present study demonstrate that efferent vagal stimulation can elicit both vasoconstrictor and vasodilator responses in the feline pulmonary vascular bed. Moreover, when the integrity of the adrenergic nerves to the lung was destroyed and vasoconstrictor tone was elevated, vagal stimulation caused marked frequency-dependent decreases in pulmonary vascular resistance. Injections of acetylcholine also dilated the pulmonary vascular bed, and responses to vagal stimulation and acetylcholine were blocked by atropine and enhanced by physostigmine. Vasodilator responses to vagal stimulation were not dependent on changes in bronchomotor tone and lung volume, or changes in aortic pressure and bronchopulmonary shunt flow. The present studies indicate that stimulation of cholinergic fibers in the vagus releases acetylcholine which acts on muscarinic receptors to dilate the pulmonary vascular bed. Studies with hexamethonium, a ganglionic blocking agent, suggest that the feline pulmonary vascular bed is well supplied with functional cholinergic terminals whose preganglionic fibers travel in the cervical vagus.

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References


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