Bronchial vasodilator pathways in the vagus nerve of dogs

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Pisarr, T. E., M. P. Zimmerman, T. E. Adrian, J. C. G. Coleridge, and H. M. Coleridge. Bronchial vasodilator pathways in the vagus nerve of dogs. J. Appl. Physiol. 86(1): 105–113, 1999.—Bronchial vasodilation in dogs is mediated largely by vagal pathways. To examine the relative contribution of cholinergic and noncholinergic parasympathetic pathways and of sensory axon reflexes to vagal bronchial vasodilation, we electrically stimulated the peripheral vago-sensory nerve in 10 chloralose-anesthetized dogs and measured bronchial artery flow. Moderate-intensity electrical stimulation (which did not activate C-fiber axons) caused a rapid voltage- and frequency-dependent vasodilation. After atropine, vasodilation was slower in onset and reduced at all voltages and frequencies: bronchial vascular conductance increased by 9.0 ± 1.5 (SE) ml·min⁻¹·100 mmHg⁻¹ during stimulation before atropine and 5.5 ± 1.4 ml·min⁻¹·100 mmHg⁻¹ after (P < 0.02). High-intensity stimulation (sufficient to recruit C fibers) was not studied before atropine because of the resulting cardiac arrest. After atropine, high-intensity stimulation increased conductance by 12.0 ± 2.5 ml·min⁻¹·100 mmHg⁻¹. Subsequent blockade of ganglionic transmission, with arterial blood pressure maintained by a pressure reservoir, abolished the response to moderate-intensity stimulation and reduced the increase to high-intensity stimulation by 82 ± 5% (P < 0.01). In 13 other dogs, we measured vasoactive intestinal peptide-like immunoreactivity in venous blood draining from the bronchial veins. High-intensity vagal stimulation increased vasoactive intestinal peptide concentration from 5.7 ± 1.8 to 18.4 ± 4.1 fmol/ml (P = 0.001). The results suggest that in dogs cholinergic and noncholinergic parasympathetic pathways play the major role in vagal bronchial vasodilation.

Cholinergic vasodilator; axon reflex; bronchial artery; vasoactive intestinal peptide

Increases in bronchial blood flow, as well as in airway smooth muscle tone, may be important factors in airway obstruction (7, 23). Indeed, airway defense reflexes, which may contribute to airway obstruction in exercise-induced asthma, evoke bronchial vasodilation along with bronchoconstriction (25, 27). Although the bronchial vasculature is influenced by sympathetic pathways (36), the reflex bronchial vasodilation is largely mediated by vagal pathways (5). However, the identity of the neurotransmitters that mediate the vagal vasodilator influences is unresolved.

In many species, vasodilator influences on the tracheal vasculature include cholinergic and noncholinergic parasympathetic influences as well as neuropeptides released from the terminals of unmyelinated (C-fiber) sensory nerves (11, 13, 20, 21, 32). However, the situation in the bronchial circulation is less clear. In pigs, vagal stimulation evokes a slowly developing bronchial vasodilation that is not reduced by atropine or ganglionic blockade; Matran et al. (21) concluded that in this species vagally mediated bronchial vasodilation depends entirely on vasodilator neuropeptides released by sensory C fibers. In contrast, little evidence of sensory C-fiber-mediated vagal vasodilation was found by Martling et al. (18) in cats treated with atropine, where cholinergic pathways per se were not studied. Noncholinergic bronchial vasodilation during vagal stimulation was abolished by ganglionic blockade, suggesting mediation by noncholinergic parasympathetic pathways. Yet another conclusion was reached by Baile et al. (2) on the basis of studies in sheep, in which bronchial vasodilation during vagal stimulation was completely abolished by atropine, implying a purely cholinergic innervation. In an early study in dogs, vasodilation to electrical stimulation of the vagus nerve was not inhibited by atropine, suggesting a completely noncholinergic innervation (4). However, reflex studies in sheep and dogs suggest that both cholinergic and noncholinergic preganglionic parasympathetic pathways are largely responsible for bronchial vasodilation (6, 25, 26).

The conflicting conclusions of these studies may stem not only from species differences but also from differences in the frequency, strength, and duration of vagal stimulation, which determine the relative activation of different classes of fiber. Moreover, interpretations are complicated by systemic hypotension resulting from the bradycardia during high-intensity stimulation in the absence of atropine and by hypotension after ganglionic blockade. In the present study, we have reexamined the bronchial vasodilator pathways in the vagus nerve of dogs by using electrical stimulation over a wide range of frequency and stimulus strengths. We avoided excessive bradycardia before atropine by using trains of narrow electrical pulses, insufficiently powerful to maximally activate preganglionic motor pathways. Such moderate stimulation appears to recruit activity mainly in small myelinated fibers (autonomic type B fibers), which comprise ~50% of vagal preganglionic motor fibers (1). After atropine, we used broader electrical pulses at higher intensities to recruit activity in C fibers and subsequently induced total ganglionic blockade to look for axon-reflex bronchial vasodilation. In this part of the study, we used a pressure compensator to prevent any reduction in arterial pressure caused by loss of sympathetic vasoconstrictor tone.

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The noncholinergic parasympathetic neurotransmitters of the airway circulation may include vasoactive intestinal peptide (VIP) and nitric oxide (3, 16, 36). Therefore, we looked for evidence that the neuropeptide VIP is released into the bronchial circulation during high-intensity vagal stimulation by using radioimmunoassay to measure spillover of VIP in the venous blood draining the bronchi. We also examined the vasodilation resulting from infusion of VIP into the bronchial artery.

METHODS

General. Dogs (12–24 kg) were given acepromazine maleate (1 mg/kg im; PromAce, Aveco); 30 min later they were anesthetized with α-chloralose (80 mg/kg iv). Supplemental doses of α-chloralose (10 mg/kg iv) were given hourly to maintain anesthesia. The trachea was cannulated low in the neck. The chest was opened through the right fifth intercostal space, and the lungs were ventilated with 50% O2 in air by a Harvard respirator (model 613), the expiratory outlet of which was placed under 3–5 cm of water. Tidal volume was calculated by a cardiotachometer (model 7P4C, Grass); base deficit was corrected by intravenously administered sodium bicarbonate solution.

Tracheal pressure was measured from a sidearm of the tracheal cannula by a Statham PR23-6G-300 strain gauge. Arterial blood pressure was recorded from a catheter inserted into the left femoral artery and was measured by a Statham P23Gc strain gauge. In some experiments, left atrial pressure was recorded from a catheter introduced through the right external jugular vein. An electrocardiogram (lead II) was recorded. Heart rate was calculated by a blood pressure signal. The signals representing tidal CO2, tracheal pressure, systemic arterial and left or right atrial blood pressure, heart rate, and blood flow in the bronchial artery (see Measurement of bronchial arterial flow) were recorded by a Grass model 7 polygraph. A cannula was introduced into the right femoral artery for later connection to a pressurized reservoir containing 2 liters of dextran to maintain arterial pressure after ganglionic blockade.

Measurement of bronchial arterial flow. The right bronchial (bronchoesophageal) artery and the aortic intercostal artery from which it arose were identified. The right bronchial artery supplies the carina and airways of the right lung; ~30% of its flow is to adjacent mediastinal tissues (4, 10). To reduce flow to nonrespiratory tissues, branches supplying the esophagus were ligated. A fine polyethylene catheter was inserted in the intercostal artery distal to the origin of the bronchial artery and advanced retrogradely until its tip was just downstream to the origin of the bronchial artery. With the intercostal artery ligated at the point of cannula insertion, solutions infused slowly through the catheter entered the bronchial circulation. The identity of the bronchial artery was confirmed, and its vascular territory was outlined, by injection of indocyanine green dye (Sigma Chemical) into the intercostal catheter. The bronchial artery was dissected free of connective tissue, and a 1-mm-ID ultrasonic transit-time flow probe (model 1R, Transonic Systems, Ithaca, NY) was placed around it. Blood flow was measured by a Transonic T206 small-animal, two-channel flowmeter. The signals representing bronchial arterial flow and systemic arterial blood pressure were fed to an electronic divider that calculated bronchial vascular conductance as bronchial blood flow per 100 mmHg mean systemic arterial pressure. Because changes in right and left atrial pressure were never more than 1–2 mmHg, they were not subtracted from mean arterial pressure in the estimate of conductance. Flow and conductance were recorded by the polygraph.

Stimulation of the vagus nerve. The right vagus nerve was freed high in the neck and transected, and the peripheral cut end was placed on a shielded bipolar stimulating electrode. In experiments in which plasma was collected for radioimmunoassay, the left vagus nerve was similarly prepared so that both nerves were stimulated simultaneously. Electrical pulses were delivered by a Grass stimulator (S88); pulse width was set initially at 0.1–0.2 ms (“narrow pulses-moderate stimulation”). In three dogs the nerve was also freed low in the neck, 3–4 cm distal to the stimulating electrode, and placed on a recording electrode. Compound action potentials were recorded by a Tektronix 5115 storage oscilloscope, the sweep of which was triggered by the stimulus. The compound action potentials indicated that when the stimulus pulse was ~0.2 ms wide the C wave was absent at all three voltages. When the pulse width was increased to 2 ms (“broad pulses-high-intensity stimulation”), the threshold for appearance of the C wave was 13 V.

Protocol. To examine cholinergic and noncholinergic vagal motor pathways, bronchial blood flow and conductance were recorded in 10 dogs during electrical stimulation with narrow pulses before and after block of postganglionic parasympathetic (mecinarinic) receptors with atropine (1–2 mg/kg iv). The effectiveness of cholinergic blockade was confirmed by an absence of the cardiac effects of vagal stimulation. In eight dogs the voltage and frequency dependence of the vasodilator effects was studied systematically by random delivery of trains of narrow pulses over the full range of voltage and frequency. One-minute trains of narrow pulses were delivered at 1, 2, 5, and 10 Hz at 7, 14, and 20 V. After each train, bronchial flow and arterial pressure were allowed to recover before the next stimulus was delivered. In the remaining two dogs, a more limited range of voltage and frequency was examined.

We also examined the effects of vagal stimulation before and after total ganglionic blockade in nine dogs. After atropine was administered, we increased pulse width to 2 ms and voltage to a maximum of 30 V. (It was not possible to examine the response to this high-intensity stimulation before atropine because of the extreme bradycardia and hypotension the stimulation produced.) The response to both moderate- and high-intensity stimulation was examined at 5 and 10 Hz before and after hexamethonium. Total ganglionic blockade was induced with hexamethonium in three doses each of 10 mg/kg iv at 10-min intervals. At this point in the experiments we connected the femoral arterial cannula to the dextran reservoir and pressurized the reservoir to maintain the dog’s arterial pressure at prehexamethonium levels. The volume of dextran transferred to the dog to accomplish this procedure never exceeded 200 ml.

Collection of bronchial venous blood. In 13 other dogs, bronchial venous blood was collected for radioimmunoassay. In dogs, most venous blood returning from the extrapulmonary airways drains into the azygous vein, rostral to the level of the seventh thoracic vertebra (9). Therefore, a catheter was introduced into the azygous vein through the right external jugular vein and its tip advanced ~2 cm from the junction with the cranial vena cava. The azygous vein was
occluded posterior to the seventh intercostal vein to exclude venous drainage from the posterior region of the chest wall. Additionally, intercostal veins entering the azygous vein proximal to the occlusion were tied close to the chest wall so that the blood collected from the azygous vein was highly enriched with bronchial venous blood. Bronchial venous blood samples were withdrawn at 1.4 ml/min from the azygous vein by an infusion-withdrawal syringe pump (model 901, Harvard).

To estimate the fraction of bronchial venous blood in the sample, we infused Evans blue dye (100 µg/ml at 1 ml/min) into the bronchial artery and calculated the bronchial artery plasma concentration (from the bronchial artery flow and hematocrit). We measured the concentration of dye in the plasma drawn from the azygous vein with a spectrophotometer (Pharmacia Ultrospec II). We also measured dye concentration in samples drawn from the caudal vena cava (which does not contain bronchial venous blood) to correct for dilution into the total plasma volume. We calculated the fraction of bronchial venous content in the azygous sample as

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\text{Fraction of bronchial venous blood} = \frac{[EB]_{BV} - [EB]_{CVC}}{[EB]_{BA}}
\]

where \([EB]_{BV}\), \([EB]_{CVC}\), and \([EB]_{BA}\) are the concentration of Evans blue dye in the bronchial venous, caudal vena cava, and bronchial arterial plasma, respectively. The bronchial venous content of the azygous vein plasma was variable, from essentially none in a few cases to virtually pure in others. On average, dye concentration in the sampled venous plasma was 47 ± 16% of that in the bronchial arterial plasma. Samples for radioimmunoassay were collected and measured only in those experiments in which the bronchial venous content was >20%; the mean dye concentration in these experiments was 55 ± 8%. In two experiments, we tested the recovery of exogenous VIP from bronchial venous plasma. We infused VIP at 1,500 fmol/min into the bronchial artery and estimated the bronchial arterial plasma concentration from the infusion rate and the bronchial artery flow. Recovered bronchial venous VIP concentration, measured by radioimmunoassay, was 38 and 36% of the estimated bronchial arterial concentration in these two dogs.

Protease inhibition. Neuropeptide recovery is limited by degradation by endogenous peptidases (14). To reduce this degradation, we infused a cocktail of protease inhibitors beginning 10 min before and continuing through the plasma collection. The cocktail contained-phosphoramidon (100 µg/ml; an inhibitor of neutral endopeptidase), captopril (40 µg/ml; an inhibitor of kininase II (angiotensin-converting enzyme)), and aprotinin (3,500 kallikrein-inactivating units/ml; an inhibitor of many serine proteases, including trypsin, chymotrypsin, kallikrein, and plasmin). The inhibitors were dissolved in boiled saline (to minimize oxidation) and infused into the bronchial artery at 0.5 ml/min by an infusion pump (model 11, Harvard).

Constant-flow perfusion of the bronchial artery. Increased VIP release might be masked by dilution into increased bronchial blood flow during vagal stimulation. Therefore, we minimized the change in bronchial blood flow during stimulation by administering atropine (1 mg/kg iv) and propranolol (1 mg/kg iv) before beginning the plasma collection. In addition, in 5 of the 11 dogs, we perfused the bronchial circulation at constant flow. To isolate the bronchial artery from the aorta, we ligated the parent intercostal artery between the aorta and the origin of the bronchial artery, and we confirmed that this halted flow in the bronchial artery. After administering heparin (250 U/kg), we established constant-flow perfusion of the bronchial artery with blood pumped (Masterflex Variable Speed, Cole-Parmer) from the femoral artery catheter through the bronchial artery catheter. The pump rate was set to equal the spontaneous bronchial artery flow measured before the intercostal was ligated.

Collection protocol. Bronchial venous blood (4 ml) was withdrawn at a constant rate of 1.4 ml/min by the infusion-withdrawal syringe pump. Samples were withdrawn under control conditions and during electrical stimulation of the peripheral vagus nerves at high intensity (1- to 2-ms pulse width, 10 Hz, 15 V). Blood samples were placed immediately into chilled and labeled EDTA tubes (to prevent coagulation), each containing the protease inhibitor aprotinin (1 trypsin inhibitor unit in 200 µl), and centrifuged for 15 min at 2,500 rpm at 4°C to separate plasma from the cellular components of the blood. The plasma fraction was removed, and its volume was recorded before acidification with glacial acetic acid (15 ml/ml). Samples were stored frozen (−20°C).

Radioimmunoassay. Before radioimmunoassay, the plasma samples were centrifuged to separate fibrous protein precipitate. The supernatant was passed through activated C18, reverse-phase Sep-Pak cartridges (Waters Chromatography Division, Milford, MA). After being washed, the Sep-Paks of 1 ml were eluted with 2 ml of 50% methanol or 0.1% trifluoroacetic acid. These eluates were freeze-dried for storage and later reconstituted in 1 ml of assay buffer (0.06 M sodium phosphate buffer at pH 7.4 with 0.1% Triton X-100 and 0.1% bovine serum albumin) before assay.

The VIP content of the reconstituted samples was measured by solution-phase radioimmunoassay. VIP was traced iodinated by chloramine-T oxidation. The resulting moniodinated VIP was purified by using high-resolution, reverse-phase high-pressure liquid chromatography. VIP antisera (V9) (15), a highly specific COOH-terminal antibody (sensitivity 0.3 fmol/tube), was added at a final dilution (1:320,000) that bound 50% of 1.5 fmol of labeled peptide in the absence of nonlabeled peptide. Assays were incubated for 7 days under equilibrium conditions. Free neuropeptide was separated from bound neuropeptide by the precipitation of the latter by the addition of polyethylene glycol (PEG) to give a final concentration of 12% PEG (mol wt 6,000) and the addition of 1 mol of the crude gamma globulin of rabbits. Supernatants were removed by using a vacuum line, and bound radioactivity was counted on a 10-well auto gamma counter (model 1277, Wallac, Turku, Finland). Sample peptide content was determined by comparison to a standard curve (0–100 fmol/tube) and converted to femtomoles per milliliters of original plasma sample. The within- and between-batch coefficient of variation were <8 and 12% respectively.

Infusion of VIP into the bronchial artery. In 13 dogs (some of which were not otherwise included in this study), we infused VIP into the bronchial artery. VIP was dissolved in the cocktail of protease inhibitors (see Protease inhibition) containing 1% bovine serum albumin at concentrations of 10 and 100 pmol/ml. In some experiments, a wider range of concentrations was used. The solutions were infused into the bronchial artery at 0.5 ml/min until a steady-state bronchial flow was achieved (~2 min). The bronchial arterial plasma concentration was estimated from the concentration of VIP infused, the infusion rate, and the bronchial artery flow, assuming a hematocrit of 0.4. Because the bronchial flow increased by varying amounts during the infusion, the final arterial plasma concentration achieved varied from animal to animal. Infusions that resulted in an estimated plasma flow between 10⁻⁸ and 10⁻⁹ M were used for analysis.

Analysis of results. Changes in bronchial blood flow and vascular conductance were calculated by comparing the con-
trol value before stimulation with the value at the initial peak of vasodilation (~15–20 s from the onset of stimulation) and with the final value at the end of the 60-s period of stimulation. Changes in heart rate and arterial pressure were calculated by comparing the control value before stimulation with the maximum change during the stimulation period. Results are expressed as means ± SE. Statistical analyses of the effect of frequency and treatment on changes in flow, conductance, arterial pressure, and heart rate and of the effect of stimulation on plasma peptide content were made by using ANOVA for repeated measures. If a significant effect was detected, individual means were compared by constructing contrasts by using SuperANOVA statistical software. Statistical significance was accepted if P < 0.05.

RESULTS

At the onset of the experimental protocol, blood flow in the right bronchial (bronchoesophageal) artery was 6.4 ± 0.9 ml/min (range 2.5–13.5 ml/min), which is similar to that measured previously in awake (10) and anesthetized (4, 25, 26) dogs. Bronchial vascular conductance was 5.8 ± 0.7 ml·min⁻¹·100 mmHg⁻¹ (range 2.5–11.7 ml·min⁻¹·100 mmHg⁻¹), and mean arterial pressure was 109 ± 2.7 mmHg (range 100–125 mmHg).

Effects of moderate stimulation. Stimulating the vagus nerve with trains of narrow pulses (0.1–0.2 ms), to recruit activity mainly in myelinated preganglionic motor fibers, caused a voltage- and frequency-dependent bronchial vasodilation (Figs. 1 and 2).

Before atropine, stimulation at the lowest voltage and frequency (7 V, 1–2 Hz) produced bronchial vasodilation in four of the eight dogs (Figs. 1 and 2). Vasodilation in three other dogs first occurred at 5–10 Hz, and in one dog, only after stimulus strength was increased to 14 V. Bronchial vascular conductance increased rapidly with the onset of stimulation, reaching a peak ~15–20 s after stimulation began and decreasing equally rapidly, especially with lower frequency trains (Fig. 1). Nevertheless, it usually remained elevated throughout the 60-s period of stimulation.

Vasodilation was more sustained and the initial peak less obvious as frequency and voltage increased (Figs. 1 and 2). At the higher strengths of narrow pulse-stimulation, the initial increase in conductance appeared to reach a maximum (Fig. 2). At these high strengths, it often rose again to a secondary peak 10–20 s after stimulation was terminated. Blood flow and conductance returned to their control levels after a further 10–20 s.

In general, especially over the lower range of voltage and frequency of narrow pulses, the cardiodepressor effects of vagal stimulation in the absence of atropine were not severe. Decreases in heart rate and arterial pressure became marked only at maximal voltage and frequency (20 V, 10 Hz; Fig. 3). Despite the marked fall in arterial pressure (and hence in the driving pressure for bronchial blood flow) evoked by this stimulation, bronchial blood flow increased significantly (from 4.9 ± 0.8 to 9.6 ± 1.7 ml/min after 60 s; P < 0.001).

After atropine was administered, the bronchovasodilator effects of vagal stimulation were absent at the lowest voltage and frequency, and the threshold frequency for an increase in bronchial vascular conductance rose to 5 Hz (Fig. 2). Moreover, in contrast to the rapid onset before atropine, vasodilation after atropine was of slow onset (Fig. 1). In some dogs, conductance fell slightly at the onset of stimulation, particularly with stimulation at high voltage and low frequency (Fig. 2). Thereafter, conductance increased progressively throughout the 60-s period of stimulation. At the higher levels of stimulation there was often a secondary rise after stimulation was terminated, and con-

![Fig. 1. Frequency-dependent bronchial vasodilator effects of moderate-intensity electrical stimulation (narrow pulses) of the cut peripheral end of the right vagus applied before (A) and after (B) muscarinic blockade and after subsequent ganglionic blockade (C). Vasodilation was measured as change in bronchial vascular conductance (Cbr), calculated as 100 × bronchial artery flow divided by arterial blood pressure (ABP). Stimuli were 7 V, 0.1-ms pulse width, delivered at (from left to right) 1, 2, and 5 Hz, respectively.](http://jap.physiology.org/academic.library.org)
ductance reached a maximum some 15–20 s later. Recovery was slower. Bronchial blood flow and conductance returned to control levels after 60–90 s.

After atropine, vagal stimulation no longer caused a decrease in heart rate and arterial pressure. Instead, a small increase in heart rate was detectable at higher frequencies of stimulation (Fig. 3). Arterial pressure did not change significantly.

Effects of high-intensity stimulation. In nine dogs given atropine, we compared the bronchial vasodilator effect of delivering high-intensity, broad-pulse (width 2 ms, sufficient to evoke a C wave in the compound action potential) stimulation before and after total ganglionic blockade. After atropine alone, vasodilator effects to high-intensity stimulation were just detectable in two dogs at 14–15 V and 5 Hz and were detectable in the
remaining dogs at 20 V and 2-5 Hz. With stimulation intensities above the threshold for vasodilation, vascular conductance rose slowly to a maximum. The effects were often prolonged, outlasting the stimulus by 90 s or more (Fig. 4).

After total ganglionic blockade with hexamethonium, the intensity of stimulation required to increase bronchial vascular conductance greatly increased. Narrow pulses had no effect (Fig. 1). The vasodilation to high-intensity stimulation had a time course similar to that before hexamethonium but was reduced by 80% (Figs. 4 and 5).

In three dogs, high-intensity stimulation of the vagus after total ganglionic blockade evoked a small increase in heart rate. Propranolol, tested in one dog, blocked the increase.

VIP spillover. In separate experiments in 13 other dogs, we measured the content of VIP-like immunoreactivity in venous blood draining from the bronchial veins into the azygous vein. High-intensity vagal stimulation (1-2 ms pulse width, 10 Hz, 15 V) increased bronchial venous VIP content in each of the dogs, the mean content increasing threefold ($P < 0.001$) (Fig. 6). Vagal stimulation also increased the content of VIP in the caudal vena caval blood of nine of the dogs, but the increase was smaller than in the bronchial venous blood ($P < 0.001$). In five of these experiments, the bronchial artery was perfused at constant flow to prevent dilution of any increased VIP release in the plasma. In the other eight animals, increases in flow were attenuated by administration of atropine; flow in these animals increased from $4.6 \pm 0.9$ to $7.3 \pm 1.6$ ml/min during stimulation. There was no systematic difference in the measured change in plasma VIP between the two groups.

Infusion of VIP into the bronchial artery. In 13 dogs, we infused VIP into the bronchial artery (5-50 pmol/min) to achieve estimated plasma concentrations of $10^{-9}$ to $10^{-8}$ M. At the onset of infusion, bronchial artery flow increased over 20-30 s and then leveled off to a stable plateau. Bronchial vascular conductance increased by $62 \pm 13\%$ ($P < 0.01$), from $7.4 \pm 1.1$ to $12.3 \pm 2.0$ ml·min$^{-1}$·100 mmHg$^{-1}$, during infusions that resulted in bronchial arterial plasma VIP concentrations in the range of $10^{-9}$ to $10^{-8}$ M. There was
considerable variability among animals in the sensitivity of the bronchial vasculature to VIP. Increases in bronchial vascular conductance ranged from 0 to 117% in response to the VIP infusions. In some animals tested with lower infusion concentrations, near-maximal vasodilations could be attained at plasma concentrations of $10^{-10}$ M (Fig. 7), whereas a few animals vasodilated only at concentrations above $10^{-7}$ M. In most animals, infusions of up to 50 pmol/min had little effect on systemic arterial pressure, which fell on average by only 2 ± 2% during infusions in this range; in only two animals did arterial pressure fall by >5% (Fig. 7).

**DISCUSSION**

The present experiments show that vagally mediated bronchial vasodilation in dogs can be mediated by at least three vagal pathways. Two were recruited with moderate stimulation and abolished by atropine or ganglionic blockade and therefore depended on activity in parasympathetic motor fibers. The third was recruited with high-intensity stimulation after ganglionic blockade and was therefore likely an example of the “antidromic vasodilation” (33) induced by activity in sensory C fibers.

Cholinergic pathway. The present experiments show clearly that cholinergic vagal pathways play an important role in bronchial vasodilation in dogs. They are recruited by trains of relatively mild stimuli that do not cause major cardiovascular depression. The cholinergic pathways are responsible for a vasodilation of rapid onset. Similarly, in sheep, the initial vasodilation to vagal stimulation is entirely cholinergic (2).

In contrast, previous vagal stimulation experiments in dogs and pigs failed to find evidence of cholinergic bronchial vasodilation, but part of the discrepancy may lie with differences in technique. Bruner and Schmidt (4) reported that the increase in bronchial flow induced by “weak” vagal stimulation in dogs was not reduced by atropine. However, the meaning of this early report is unclear because changes in vascular conductance were not reported. It is likely that decreases in perfusion pressure, secondary to vagally induced bradycardia, obscured the cholinergic vasodilation. Indeed, inhibition of acetylcholine breakdown with physostigmine lowered the threshold for vagal vasodilation, suggesting a cholinergic pathway.

In pigs, atropine had no effect on stimulation-induced vasodilation (21). However, these results were based primarily on low-frequency (2-Hz), high-intensity stimulation. Although high-intensity stimulation activates all fiber types, myelinated fibers typically require higher frequency than do C fibers to produce equivalent effects. For example, Widdicombe (35) recorded an average resting discharge frequency of 4.2 impulses/s in vagal motor fibers supplying the cat bronchi and reported that frequencies increased severalfold with reflex excitation. Most of the fibers studied by Widdicombe were autonomic B fibers (mean conduction velocity 9.7 m/s), the kind likely to be recruited by the narrow pulses in the present experiments. We found that the bronchial vasodilation induced by narrow pulses is frequency dependent, and in some dogs a frequency of 2 Hz had no vasodilator effect. In the few experiments in pigs in which higher frequencies were used, atropine doubled the increase in flow (21), suggesting that cardioinhibition prevented the measurement of vasodilation during the short (24-s) period of stimulation.

Noncholinergic parasympathetic efferents. After atropine administration, moderate stimulation produced a bronchial vasodilation that developed slowly. Noncholinergic bronchial vasodilation increased considerably when trains of broad pulses were used to recruit C fibers. The greater part of the increase appeared to be due to recruitment of activity in the nonmyelinated preganglionic motor fibers of the vagus, described by Agostoni et al. (1). These vasodilator effects were greatly diminished after total ganglionic blockade and hence are probably equivalent to the noncholinergic parasympathetic vasodilation described in the bronchial circulation of cats (18, 19) and the tracheal circulation of dogs (13).

Differences in technique as well as species may have contributed to the conflicting conclusions as to the existence of noncholinergic parasympathetic vasodilation reported in other species. Bronchial vasodilation to

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**Fig. 7.** Dose-dependent bronchial vasodilation to infusion of VIP into bronchial artery. VIP was dissolved in a cocktail of protease inhibitors and infused at 0.5 ml/min at indicated concentrations. Cocktail alone was infused in the control period. Infusion of 0.25, 0.5, and 50 pmol/min resulted in estimated bronchial arterial plasma VIP concentrations of $4 \times 10^{-3}$, $8 \times 10^{-3}$, and $7 \times 10^{-3}$ M, respectively. Effect of VIP on bronchial artery flow ($Q_{br}$) became nearly maximal during 0.50 pmol/min infusion; $C_{br}$ increased further during infusion at 50 pmol/ml because $Q_{br}$ was maintained despite the fall in perfusion pressure (ABP). Sensitivity of both bronchial and other systemic vessels to VIP was greater than average in this dog.
very short (3- to 20-s) bursts of moderate-intensity, moderate-frequency (8-Hz) vagal stimulation in sheep was abolished by atropine (2). This is consistent with our results, because we found that the initial peak of vasodilation to 5-Hz stimulation was abolished by atropine. However, continuing the stimulation over 60 s revealed the more slowly developing, noncholinergic vasodilation. In pigs, vagal stimulation evoked a slowly developing bronchial vasodilation that was not reduced by atropine. Although ganglionic blockade considerably attenuated and shortened the increase in bronchial flow, Matran et al. (21) concluded that the vasodilation was due entirely to release of vasodilator peptides from capsaicin-sensitive sensory nerves. However, hypotension and bronchial vasodilation after ganglionic blockade considerably complicated the interpretation, which was based on comparison of the peak percent change of vascular resistance. The data could equally be interpreted to indicate a noncholinergic parasympathetic component. Nonetheless, species differences may also contribute, because pigs have little VIPergic innervation of bronchial vasculature (20), in contrast to other mammalian species, including dogs and humans (8, 34).

Noncholinergic parasympathetic neurotransmitters may include VIP (13, 31), nitric oxide (16), and peptide histidine isoleucine, which is colocalized with VIP but is 40-fold less potent as a vasodilator (17, 30). In the present experiments, vagal stimulation increased the spillover of VIP-like immunoreactivity into the bronchial venous drainage, confirming that VIP is indeed released by vagal nerves to the airways. Although this is consistent with the suggestion that such pathways mediate the noncholinergic parasympathetic vasodilation, confirmation must await the availability of specific VIP-receptor antagonists. VIP-containing nerves of the airways also innervate airway smooth muscle and submucosal glands in dogs (8), and these nerves undoubtedly also contribute to the spillover into the bronchial veins during vagal stimulation. Vagal stimulation also increased VIP spillover into blood draining in the caudal vena cava, undoubtedly reflecting the important vagal VIPergic innervation of the gut.

Although our results do not allow us to estimate the contribution of VIP to the vasodilation, we confirmed that VIP in the range from $10^{-8}$ to $10^{-9}$ M increased bronchial vascular conductance by 60%. This appears to be roughly comparable to the 40% increase in tracheal vascular conductance after bolus injection of $10^{-9}$ mol in dogs (12), although the comparison is complicated by the difficulty of estimating the plasma concentration achieved by the bolus injection. Similarly, conduit bronchial arteries of pigs studied in vitro were dilated ~20% by VIP concentration in the range from $10^{-8}$ to $10^{-9}$ M (22). This concentration range is higher than the venous plasma concentrations in the range of $10^{-11}$ M we measured by radioimmunoassay. However, the measured venous plasma concentration only qualitatively reflects the release of VIP at motor nerve-vascular smooth muscle synapses in the media of the bronchial vasculature, because the peptide is both degraded and diluted in traveling from the synapse to the collection point in the azygous vein.

Axon reflexes in sensory nerves. In the present experiments, high-intensity stimulation of the vagus nerve continued to evoke some bronchial vasodilation after total ganglionic blockade. This vasodilation, likely mediated by release of neuropeptides from the terminals of sensory C fibers, accounted for only 20% of the noncholinergic vasodilation. This pathway appears to correspond to the capsaicin-sensitive pathway said to account for vagal vasodilation in the bronchial circulation of pigs (21). The present neuropeptides released by sensory C fibers are believed to include neurokinin A, substance P, and calcitonin gene-related peptide (CGRP), all of which are vasodilators (36). The present results give no insight into which of these neurotransmitters mediate the hexamethonium-resistant vasodilation we observed. However, we have recently reported that the highly specific CGRP-receptor antagonist CGRP 8-37 reduces atropine-resistant vasodilation to high-intensity stimulation of the vagus nerve of dogs (24).

Vagal vasoconstrictor fibers. In some atropinized dogs, a minor degree of bronchial vasoconstriction at the onset of vagal stimulation often preceded the more gradual vasodilation. At higher stimulation strengths, a secondary vasodilation often occurred when stimulation was terminated, suggesting release of a vasoconstrictor mechanism. Sympathetic vasoconstrictor effects on the bronchial circulation are well known (4, 28, 36); thus the most likely explanation of the vasoconstrictor effects in the present study is stimulation of sympathetic fibers coursing with the vagus nerve before eventually reaching their thoracic destination (1, 29). These fibers also likely accounted for the tachycardia that occurred during stimulation after atropine. Another possibility is suggested by the paradoxical increase in both bronchial artery flow and conductance after cholinergic-receptor blockade in awake dogs reported by Hennessey et al. (10). This observation suggests a tonic cholinergic vasoconstrictor influence on the bronchial circulation in conscious dogs; however, the mechanism by which peripheral cholinergic innervation could be responsible for vasoconstrictor tone is unclear.

In summary, our results demonstrate that vagal bronchial vasodilation in dogs is largely mediated by efferent ganglionic pathways that include both cholinergic and noncholinergic parasympathetic neurotransmitters. Although we did not identify the noncholinergic neurotransmitters, we demonstrated the release of VIP into the bronchial circulation during vagal stimulation, implicating this neuropeptide as a candidate. Nonganglionic pathways, possibly in sensory nerve axons, also exist but make a smaller contribution. The present evidence does not indicate which of the three vagal vasodilator pathways predominates during physiologic vasodilation. However, reflex experiments in dogs and sheep in which sensory C fibers in the bronchi were stimulated by capsaicin (6, 26) or water (25) suggest that parasympathetic motor pathways play the major
role. Sensory or axon-reflex vasodilation plays a part in some animals, more often with more powerful sensory stimuli such as capsaicin (5).

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