Reflex tracheal smooth muscle contraction and bronchial vasodilation evoked by airway cooling in dogs

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Pisarri, Thomas E., and Gordon G. Giesbrecht. Reflex tracheal smooth muscle contraction and bronchial vasodilation evoked by airway cooling in dogs. J. Appl. Physiol. 82(5): 1566–1572, 1997.—Cooling intrathoracic airways by filling the pulmonary circulation with cold blood alters pulmonary mechanoreceptor discharge. To determine whether this initiates reflex changes that could contribute to airway obstruction, we measured changes in tracheal smooth muscle tension and bronchial arterial flow evoked by cooling. In nine chloralose-anesthetized open-chest dogs, the right pulmonary artery was cannulated and perfused; the left lung, ventilated separately, provided gas exchange. With the right lung passively ventilated, filling the right pulmonary circulation with 5°C blood increased smooth muscle tension in an innervated upper tracheal segment by 23 ± 6 (SE) g from a baseline of 75 g. Contraction began within 10 s of injection and was maximal at ~30 s. The response was abolished by cervical vagotomy. Bronchial arterial flow increased from 8 ± 1 to 13 ± 2 ml/min, with little effect on arterial blood pressure. The time course was similar to that of the tracheal response. This response was greatly attenuated after cervical vagotomy. Blood at 20°C also increased tracheal smooth muscle tension and bronchial flow, whereas 37°C blood had little effect. The results suggest that alteration of airway mechanoreceptor discharge by cooling can initiate reflexes that contribute to airway obstruction.

Methods

General. Dogs (20–25 kg) were given acepromazine maleate (1 mg/kg im; PromAce, Aveco); 30 min later they were anesthetized with α-chloralose (80 mg/kg iv). Supplementary doses of α-chloralose (10 mg/kg iv) were given hourly to maintain surgical anesthesia.

The trachea was cannulated low in the neck with a split-lumen tube (Kottmaier). The left lung, which served as the gas exchanger, was ventilated separately with 50% O2 in air by a dual Harvard pump supplying a tidal volume of 15 ml/kg at a frequency of 15–20 cycles/min. Positive end-expiratory pressure was set at 3–4 cmH2O. The right lung was connected through the cannula to a three-way valve that allowed either ventilation with 5% CO2 in air or inflation from a static air pressure source. Right and left airway pressures were measured by Statham PR23–6G–300 strain gauges. Percent CO2 in the expired gas (left lung) was monitored continuously by a Noremco 200 respiratory gas monitor (Datex); end-tidal Pco2 was kept at ~35 Torr by adjustment of the ventilator rate. Arterial blood samples were withdrawn periodically, and blood-gas and acid-base values were determined with an automatic analyzer (model 175, Corning); base deficit was corrected by intravenous administration of sodium bicarbonate solution.

We perfused the right lung in situ as previously described (16, 17). The chest was opened in the right fifth intercostal space. Heparin (250 U/kg) was administered as a bolus; supplementary doses were administered hourly. The right pulmonary artery was isolated inside the pericardium, cannu-

IN ASTHMATIC INDIVIDUALS, hyperventilation, particularly with cold dry air, can result in airway obstruction (10). However, despite many human and animal studies, neither the transduction mechanism of the cold, dry stimulus nor the effector mechanism that produces the obstruction has been clearly identified. Most explanations of the initiating stimulus focus on either the direct cooling effect of the inspired air or on the increased osmolarity of the airway surface liquid that accompanies humidification of the inspired air (12). The relative importance of these two components of the stimulus has been difficult to separate because, on the one hand, cold air necessarily is dry and, on the other hand, the evaporation that occurs during ventilation with even warm dry air inevitably cools the airway.

The pathways by which either drying or cooling causes obstruction remain uncertain. Increasing osmolarity of the airway lining has been hypothesized to act through stimulation of afferent nerves (23, 27) or through release of mediators from airway mast cells (3), both of which can induce bronchoconstriction, bronchial vasodilation, plasma extravasation, and mucus secretion. Airway cooling has been hypothesized to act locally on the airway vasculature to cause vasoconstriction followed by reactive hyperemia that narrows the lumen (20). However, this may not be the sole mechanism by which cooling produces obstruction. Localized tracheal cooling, without drying, reduces external tracheal diameter, implying tracheal smooth muscle contraction rather than submucosal engorgement (11). Moreover, airway cooling, even in the absence of drying, alters the discharge of lower airway mechanoreceptors (16), an effect potent enough to alter the pattern of breathing (17). Because these same airway mechanoreceptors influence airway smooth muscle tone, cooling could produce obstruction through neural mechanisms (25).

Therefore, in the present study, we measured the effect of cooling the intrapulmonary airways on tracheal smooth muscle tension and bronchial arterial flow before and after eliminating neural pathways by cutting the vagus nerves. To avoid concomitant drying of the airway, we cooled the airways of the right lung by filling the right pulmonary vessels with cold blood. Because the effect of cold on afferent discharge depends on lung volume (16), we examined the responses to cooling both during phasic ventilation and with the right lung held at both low and high constant volumes.

METHODS
lated peripherally, and perfused with blood from a cannula in the right femoral artery. Right pulmonary arterial pressure and blood temperature were monitored by a Swan-Ganz catheter, cut just distal to the temperature probe, inserted through a sidearm into the pulmonary arterial cannula. Right pulmonary arterial pressure was kept at \( \approx 20 \) mmHg by adjusting a variable resistance on the perfusion circuit. A heat exchanger in the perfusion circuit maintained blood temperature at 37–38°C. Test boluses of blood were injected through a second sidearm located upstream to the first.

Systemic arterial pressure was measured from a catheter in the left femoral artery by a Statham P23Gc strain gauge. An electrocardiogram (lead II) was recorded. Heart rate was measured by a cardiotachometer (model 7P4C, Grass) triggered by the arterial pressure signal. All signals were recorded by a Grass polygraph (model 7).

Measurement of bronchial arterial flow. The right bronchial artery and the aortic intercostal artery from which it arose were identified. A fine polyethylene catheter was inserted in the intercostal artery distal to the origin of the bronchial artery. The catheter was advanced retrogradely and tied in place with its tip just downstream to the origin of the bronchial artery so that solutions injected slowly through the catheter entered the bronchial circulation. The identity of the bronchial artery was confirmed and its vascular territory outlined by injection of cardiogreen dye into the intercostal catheter. Small branches supplying the esophagus were ligated. An ultrasonic transit time flow probe (1-mm ID; model 1R, Transonic Systems, Ithaca, NY) was placed on the bronchial artery, and blood flow in the artery was measured by a Transonic T206 small-animal flowmeter.

Tracheal smooth muscle tension. A segment of trachea (4–5 cm) immediately caudal to the larynx was incised ventrally in the midline and transversely across both ends of the midline incision. The posterior wall was left intact. Each midline cut edge was retracted laterally by nylon threads attached to a stationary bar on one side and to a force-displacement transducer (model FT03, Grass) on the other. The segment was stretched to a baseline tension of 75 g. All responses were measured as change from this baseline. Threads were placed around the recurrent and pararecurrent laryngeal nerves to allow transection before cervical vagotomy (see Protocol). We only performed experiments in preparations in which the viability of the preparation was confirmed by the previously characterized reflex contraction of the tracheal smooth muscle after injection of capsaicin (10 µg/kg) into the pulmonary circulation (9).

Protocol. We examined the effect of cooling the right pulmonary blood under two conditions: 1) during cyclic positive-pressure ventilation of the right lung and 2) with the right lung maintained at a constant inflation pressure of 5 or 15 cmH₂O. The pulmonary blood temperature was changed by injection of boluses of blood having a volume (1.25 ml/kg plus the dead space of the tubing) that was previously found to fill the right pulmonary vasculature without entering the left atrium (17). At least 10 min before each series of tests, three boluses of blood were withdrawn from the femoral artery into separate syringes and replaced by 6% dextran. The syringes were placed in water baths at 5, 20, and 37°C. Before each bolus of blood was injected, flow to the right pulmonary artery was stopped and control tracheal smooth muscle tension and bronchial arterial flow were recorded for 60 s (Fig. 1). A bolus was then injected over \( \approx 10 \) s. Tension and flow were recorded for 60 s from the start of injection, after which right pulmonary arterial perfusion was restored. The blood boluses of different temperatures were injected in random order. Filling the right pulmonary circulation with 5
and 20°C blood lowers the right lung temperature to ~30 and
33°C, respectively (16, 17).

To examine the effect of lung volume on the response to
cooling, we injected blood at 5, 20, and 37°C while the static
inflation pressure of the right lung was set, in random order,
at 5 and 15 cmH₂O. In these experiments, pulmonary arterial
perfusion was stopped only after tension and flow had stabili-
zied after the step change in airway pressure.

Finally, in seven of the dogs, injection of 5°C blood was
repeated after the cervical vagus nerves were cut bilaterally.
The effect of vagotomy on the bronchial arterial response
was measured in each of these dogs. The effect on the tracheal
smooth muscle response was examined only in the six dogs in
which a response to cold blood remained after we cut the
recurrent and pararecurrent laryngeal nerves, which supply
some of the motor innervation of tracheal smooth muscle but
are interrupted by cervical vagotomy. After the recurrent
nerves were cut, the motor innervation of the trachea (from
the superior laryngeal nerves) is not further reduced by
subsequent cervical vagotomy; therefore, elimination of the
response could be attributed solely to interruption of vagal
afferents.

Analysis of results. Control tension and flow were mea-
sured with the right pulmonary arterial perfusion stopped
over the 30 s preceding each injection. The response to
injection was measured as the maximal change in the period
between injection and the restoration of vascular perfusion.
Results are expressed as means ± SE. Statistical significance
of the responses, comparisons of the response to blood at
different temperatures, and comparisons to injection of cold
(5°C) blood at different airway pressures were made by using
analysis of variance (ANOVA) for repeated measures. If a
significant effect was detected, individual means were com-
pared by constructing contrasts by using SuperANOVA statis-
tical software. If the data were not normally distributed, a
nonparametric test (Wilcoxon signed-rank test) was used.
Statistical significance was accepted if P < 0.05.

RESULTS

Lung phasically ventilated. In nine dogs, we mea-
sured the changes in tracheal smooth muscle tension
and, in eight of these, bronchial arterial flow (in 1 dog,
no bronchial artery could be identified) in response to
filling the right pulmonary circulation with blood at 5,
20, and 37°C. With both lungs phasically ventilated,
cessation of right pulmonary arterial flow generally
caused a small increase in bronchial arterial flow and
small, variable changes in tracheal smooth muscle
tension. Within 10 s of the filling of the right pulmonary
circulation with 5 or 20°C blood, tracheal smooth
muscle tension and bronchial arterial flow began to
increase, reaching a peak within 30 s. Both tension
and flow remained near their peak values for 30 s
and returned to control only after the pulmonary perfusion
(with body temperature blood) was restored (Fig. 1).

The increases in tension and flow were accompanied in
most experiments by a small increase in peak airway
pressure (0.2–1.2 cmH₂O) of both the left and right
airways, which was maximal 30–40 s after the injection
of cold blood (Fig. 1).

The response of tracheal tension to cooling the pulmo-

Fig. 2. Average change (with SE) of Ttr (∆Ttr) from baseline caused
by filling right pulmonary circulation with blood at indicated tempera-
tures in 9 dogs during phasic ventilation of right lung. ○, Control
averaged over 60 s before injection; ●, peak response. Response
significantly different from response to 37°C blood: *P < 0.05; **P <
0.01.

Fig. 3. Average (with SE) Qbr before (○) and at peak response to (●)
filling right pulmonary circulation with blood at indicated tempera-
tures in 8 dogs during phasic ventilation of right lung. Control was
averaged over 60 s before injection. Response significantly different
from response to 37°C blood: **P < 0.01; ***P < 0.001.
The increase in static lung volume often caused a transient decline in bronchial blood flow that recovered within 1 or 2 min. Injection of either 5 or 20°C blood significantly increased bronchial blood flow at both high and low lung volumes (Figs. 4 and 6). At the higher lung volume, the vasodilation evoked by injection of 5°C blood was slightly smaller than at the lower volume ($P < 0.02$; Fig. 6).

Injection of 37°C blood had no effect on either tension or flow at either lung volume.

Effect of vagotomy. Bilateral cervical vagotomy abolished the tracheal smooth muscle contraction and greatly reduced the bronchial vasodilation (Figs. 7 and 8). Cutting the cervical vagus nerves to interrupt afferent conduction from the lungs abolished the bronchoconstriction in each of six dogs in which a tracheal smooth muscle response remained after the recurrent laryngeal nerves were cut. The effect of vagotomy on the reflex vasodilation was more variable. In two dogs, vagotomy abolished the bronchial vasodilation; in the five others, it was greatly attenuated (to $38 \pm 5\%$ of the prevagotomy response).

DISCUSSION

Cooling the lower airways by decreasing the temperature of pulmonary arterial blood, thereby avoiding changes in the osmolarity of the airway surface liquid, increased both airway smooth muscle tension and bronchial blood flow. The intensity of the effect was proportional to the degree of cooling; injection of blood at 5°C caused the greatest changes, blood at 20°C caused smaller changes, and blood at body temperature had no effect. The evidence suggests that the tracheal smooth muscle contraction and most of the bronchial vasodilation were due to a reflex having an afferent limb that traveled in the vagus nerve.

Tracheal contraction. In the case of the tracheal smooth muscle, only a reflex effect is possible. The isolated tracheal segment itself was not cooled by the cold blood, which remained in the right pulmonary circuit throughout the response. Communication between the cooled lower airway and the tracheal smooth muscle could occur only through neural pathways passing through the central nervous system to the parasympathetic efferents to the trachea. The afferent pathway is in the vagus nerve, because bilateral cervical vagotomy abolished the response; the efferent parasympathetic pathway through the superior laryngeal nerve remains intact after cervical vagotomy.

Reflex smooth muscle contraction could be initiated by stimulation of rapidly adapting pulmonary stretch receptors, by reduced discharge of slowly adapting pulmonary stretch receptors, or by both. Although stimulation of the afferent endings of vagal sensory C fibers in the lung and airways evokes tracheal smooth muscle contraction (9), these afferents are not significantly affected by injection of cold blood (16) and hence are unlikely to contribute to the cold-evoked response. Rapidly adapting receptors are stimulated by injection
of cold but not of warm blood, and the stimulation is enhanced at higher lung volume (16). These receptors are widely believed to initiate reflex airway contraction (8, 24, 28), although the interpretation of the evidence is controversial (7, 8). Thus cold-induced tracheal smooth muscle contraction could be initiated by rapidly adapting receptor stimulation.

Cold-blood injection might also cause tracheal smooth muscle contraction by reducing the afferent activity of slowly adapting receptors, which inhibit the vagal bronchomotor neurons responsible for resting airway smooth muscle tone (8, 28). During phasic ventilation, the injection of cold blood reduces transmission of slowly adapting receptor input to the central nervous system (16). The net effect of cold on slowly adapting receptors results from the interaction of two opposing influences: cold reduces the maximal frequency of axon discharge but also directly stimulates the receptors. At high lung volumes, when the discharge frequency of most slowly adapting receptors is high, the axonal effect predominates and reduces input from these receptors. At low lung volumes, the opposing effects may cancel out, producing no net change in slowly adapting stretch receptor input (16). In the present experiments, holding the right lung at high volume enhanced the airway smooth muscle response to cold in most animals compared with holding the lung at low volume, a result consistent with mediation by inhibition of slowly adapting receptor activity. However, because baseline smooth muscle tone is reduced by the increased inhibition of vagal bronchoconstrictor efferents at the higher lung volume, the enhanced response may be influenced by changes in the resting muscle length and, therefore, may not exclusively reflect increased inhibition of the slowly adapting receptor input. Moreover, because the stimulation of rapidly adapting receptors by cold is also enhanced at high lung volumes (16), we cannot estimate the relative contribution of the two receptors to the tracheal smooth muscle response.

Although cooling confined to the central airways can evoke airway smooth muscle contraction (11, 29), contraction elicited by cooling the intrathoracic airways has not previously been demonstrated. Indeed, perfusion of the left lower lobe with 30°C blood for 2 min had little direct effect on peripheral airway resistance (14) and actually attenuated the increase in peripheral airway resistance evoked by cold airflow or hypertonic saline aerosol but not the direct effect of histamine aerosol (13, 15). The design of the above-mentioned studies by Freed and colleagues (13–15) makes them more likely to detect local effects of cold, whereas the present study was optimized to detect reflex effects. In the studies of Freed and colleagues, cooling was confined to a single lung lobe maintained at low distending pressure. Both the limited area of cooling and the low baseline mechanoreceptor discharge (at the low lung volume) would limit the effect of cooling on mechanoreceptor afferent input. In contrast, our study directly cooled an entire lung at normal volume. Second, Freed et al. measured resistance in airways that were directly cooled, whereas we measured the contraction of tracheal smooth muscle that was not cooled (because it
was perfused by the body temperature blood of the systemic circulation. Because cooling reduces the contractile response of tracheal (4) and bronchial (19) smooth muscle to cholinergic agonists or electrical field stimulation in vitro, reflex contraction in the lower airway could be opposed by direct cooling. Nonetheless, the small but consistent increase in peak right airway pressure we observed suggests that contraction of the airway smooth muscle did occur in the cooled lobe. Finally, because Freed et al. (22) cut cervical vagal nerves, we do not rule out the possibility that the stimulus was delivered to only one lung, which has been shown to be less prominent than in our experiments, in which the stimulus was delivered to both lungs. After vagotomy, which removed the reflex component of the vasodilation, we did not observe an unmasked local reflex pathway. The attenuation indicates that the afferent or efferent reflex pathways, or both, travel in the vagus nerve.

The identity of the vagal afferents that might mediate the reflex vasodilation is uncertain. As discussed earlier (see Tracheal contraction), the discharge of both slowly and rapidly adapting pulmonary stretch receptors, but not pulmonary C fibers, is altered by cooling (16); however, there is little evidence for a reflex effect of either type of mechanoreceptor on bronchial vascular resistance. A bronchial vasodilator effect of slowly adapting receptor stimulation is suggested by the observation that injection of water in the airway, a strong stimulus to slowly adapting receptors (23), evokes reflex bronchial vasodilation (22). However, the role of rapidly adapting receptors in the water-evoked vasodilation cannot be confirmed because water also stimulates lung and airway C fibers (23). If rapidly adapting receptor stimulation alone were responsible for the cold-evoked vasodilation, we would have expected enhanced vasodilation at high static airway pressure, which increases the stimulation of rapidly adapting receptors by cold blood (16). This did not occur; indeed, the vasodilation was somewhat reduced at higher pressures. However, increasing airway pressure exerts physical effects on the bronchial vessels that may oppose any reflex vasodilation (1). An additional possibility is that metabolic vasodilation, secondary to bronchial smooth muscle contraction, contributes to the vagally dependent vasodilation. Thus the exact vagal pathways that mediate the vasodilation remain uncertain.

The mechanism by which hyperventilation of dogs with cold or dry air increases tracheal and bronchial blood flow (5) has never been satisfactorily resolved (26). Airway drying was felt to be more important than airway cooling in the hyperventilation-evoked vasodilation (5), and previous experiments have provided little evidence of cold-evoked reflex airway vasodilation in dogs in the absence of drying. Instillation of cold isotonic saline into the trachea of dogs increased tracheal vascular conductance by only 6% (11), in contrast to the 75% increase in bronchial vascular conductance in the present study. We do not know whether the difference resulted from the different means of delivering the cold stimulus or from a difference between tracheal and bronchial circulation. In a study of the lower airway vasculature, perfusion of the isolated pulmonary circulation to a single lung lobe with cold blood decreased systemic (i.e., bronchial) flow to the lobe (2). Because the cold stimulus and the flow measurement were confined to the same lobe, reflex effects would be less prominent than in our experiments, in which the stimulus was delivered to only one lung while we measured the entire bronchial arterial flow. However, a difference between local and reflex effects cannot fully account for the differences between the studies.

After vagotomy, which removed the reflex component of the vasodilation, we did not observe an unmasked local vasoconstriction. The difference may be in part due to the higher sensitivity of our protocol to acute, rapid effects. Whereas we measured flow continuously in response to 1-min decreases in temperature, the earlier study (2) averaged flow in 5-min intervals over 30 min of cooling.

Relevance to the airway defense response and hyperventilation-induced airway obstruction. The neural reflexes we have described are undoubtedly important in protecting the lower airways. Physiologically, airway cooling signals the presence of air that has not been completely conditioned. Bronchoconstriction and bronchial vasodilation, by simultaneously reducing ventilation and increasing mucosal blood flow, restore the balance between inspiratory airflow and availability of heat and moisture to condition it. Cooling might also signal the presence in the airway of an exogenous, potentially noxious, substance, because an inhaled liquid or solid would not be warmed as rapidly as air. Bronchoconstriction would limit the entry of the potential irritant while...
increased bronchial flow would be the first step in an inflammatory process to neutralize the irritant and repair any damage it produced.

In hypersensitive individuals, these same reflex pathways could contribute to hyperpnea- or cold-induced airway obstruction. Filling the right pulmonary circulation with 5 and 20°C blood lowers the right lung temperature to ~30 and 33°C respectively (16, 17), temperatures that have been reported in the walls of subsegmental bronchi during hyperventilation with cold air (18). Thus, even in the absence of drying, temperatures that provoke airway obstruction in asthmatic individuals can evoke reflex bronchoconstriction and vasodilation, both of which contribute to airway obstruction. Pisarri et al. (21–23) have previously shown that changes in airway osmolarity, in the absence of temperature change, evoke similar reflex responses mediated by stimulation of airway and pulmonary C fibers and rapidly adapting receptors. Together, these studies suggest that regardless of whether airway cooling or airway drying initiates the airway obstruction, reflexes elicited by airway afferents may contribute. Clearly, these mechanisms by themselves do not explain hyperpnea- or cold-induced airway obstruction. Because both the cold-sensitive and osmolality-sensitive reflex pathways were demonstrated in mixed-breed “nonasthmatic” dogs, the mere presence of the reflex pathways is not sufficient to cause obstruction. The observation that exercise-induced airway obstruction in susceptible individuals occurs only after the end of exercise suggests that the abnormality involves an imbalance between obstructive pathways, such as those we have demonstrated, and protective mechanisms, such as the bronchodilating effects of circulating catecholamines. While obstructive and protective mechanisms have a similar time course in normal individuals, asthma may result from prolongation of the obstructive influences beyond the time course of the protective influences.

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