Airway cooling: stimulus specific modulation of airway responsiveness in the canine lung periphery

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ABSTRACT: We used a wedged bronchoscope technique in conjunction with an in situ isolated perfused left lower lobe preparation in anesthetized dogs to examine cold-associated airway modulation of peripheral lung responses to dry airflow, hypocapnia, and aerosols of histamine and hypertonic NaCl. In this preparation, airway wall temperature was rapidly lowered by decreasing the temperature of blood perfusing the wedged sublobar segment. Cooling significantly attenuated responses to dry air, hypertonic NaCl aerosol, and hypocapnic challenge. In contrast, cooling did not affect peripheral lung responses to aerosolized histamine. Thus, cooling per se does not inhibit the responsiveness of smooth muscle.

We conclude that, depending on the stimulus, cooling can modulate airway reactivity. We speculate that cooling attenuates hypocapnia, hypertonic aerosol, and dry air-induced bronchospasm via a cold induced reduction in neuronal activity or mediator production and release.

The controversy surrounding the stimulus that initiates exercise-induced asthma has persisted for over a decade, and the mechanism responsible for this type of airflow-induced bronchoconstriction (AIB) remains unknown. Most investigators believe that either airway cooling or airway drying initiates AIB, and several hypotheses each rooted in this central idea have evolved. Some investigators believe that airway cooling triggers bronchoconstriction in asthmatic subjects, and a rewarming-induced hyperaemia and oedema results in airway obstruction [1, 2]. Others suggest that changes in airway fluid osmolality resulting from evaporative water loss initiates mediator release and triggers AIB [3, 4].

We hypothesize that AIB results from an imbalance between two opposing mechanisms: an excitatory pathway stimulated by airway drying and an inhibitory pathway initiated by airway cooling [5, 6]. This hypothesis was based on data obtained from a canine model of exercise-induced asthma [5, 7] that are remarkably similar to those reported for human asthmatics [1]. A comparison of these human and canine data suggests that airway cooling does not initiate bronchoconstriction, but attenuates AIB.

Exercise, hyperventilation of dry air, and inhalation of hypertonic aerosols produce similar physiological responses in most asthmatic individuals [8-11], suggesting that some excitatory pathways may be shared. Unlike the "cooling/rewarming" hypothesis, the drying related hypotheses can account for these similarities by evoking hyperpnoea or hypertonic aerosol-induced changes in the intrapulmonary osmotic milieu. If drying alters periciliary, paracellular, or intracellular fluid osmolality to initiate AIB, and if cooling antagonizes this response, then hypertonic aerosol and dry air induced responses should be antagonized by airway cooling in a similar fashion.

Hypertonic aerosol-induced bronchoconstriction (HIB) in canine peripheral airways, like AIB, is analogous to that seen in human asthmatics. Significant correlations have been demonstrated between AIB and HIB in both asthmatic individuals [4, 8, 11, 12] and our canine model [13]. In this paper, we examine the effect of cooling on peripheral airway responses to dry air and hyperosmotic aerosol challenges, and to two additional nonspecific bronchoactive agents. We demonstrate that cooling attenuates peripheral lung constriction to dry air, hypertonic NaCl aerosol, and hypocapnia, but does not alter responses to aerosolized histamine. We conclude that 1) cooling does not directly affect smooth muscle activity, 2) the modulation of airway responsiveness via cooling is stimulus dependent, and 3) cold-inhibition of AIB and HIB is
consistent with the idea that exercise-induced asthma is a result of an imbalance between the stimulatory effects of airway drying and the inhibitory effects of airway cooling.

**Materials and methods**

Dogs were handled in accordance with the standards established by the USA Animal Welfare Acts set forth in DHEW (NIH) guidelines and the Policy and Procedures Manual published by the Johns Hopkins University School of Hygiene and Public Health's Animal Care and Use Committee. Male mongrel dogs (mean weight±SE=18.9±0.7 kg, n=22) were anesthetized with pentobarbital sodium (30 mg·kg⁻¹). A tracheotomy was performed, a dual portal tracheal tube inserted, and the dog was ventilated using a Harvard constant volume respirator. Anaesthesia was supplemented with 30 mg of pentobarbital sodium and 1 mg of pancuronium bromide every 30 min throughout the experiment. End-expiratory CO₂ was monitored with a CO₂ analyser (Beckman LB-2) and maintained between 4–5% by adjusting respirator frequency. Rectal temperature was monitored and maintained with a warming pad during the course of the experiment. Heart rate (HR) and mean arterial pressure (MAP) were monitored throughout all experimental trials via a catheter placed in the femoral artery.

**Measurement of peripheral airway response in the canine lung periphery**

A fiber-optic bronchoscope (Olympus BFA-4B2, 5.5 mm OD) was visually guided into an in situ isolated perfused sublobar segment in the left lower lobe (LLL). Room temperature 5% CO₂ in air was delivered to the wedged segment at a constant flow rate (200 ml·min⁻¹) through one lumen of a double lumen catheter that was inserted through the suction port of the bronchoscope. The other lumen was used to monitor pressure at the tip of the scope (P[subl]=sublobar pressure). With ventilator stroke and frequency fixed, end expiratory P[subl] reflects airway resistance or airway tone in the canine lung periphery.

**In situ isolated lobe technique**

Blood flow to the LLL was controlled using an open chest preparation that was previously described in detail [5]. Briefly, a catheter was placed in the branch of the pulmonary artery (PA) entering the LLL. Lobar arterial pressure (P[suba]) and temperature (T[suba]) were measured at the tip of the PA catheter via a modified thermocatheter catheter. Blood used to perfuse the LLL was taken from the femoral vein and was passed through a rotary pump set at 150 ml·min⁻¹, a bubble trap, and a heat exchanger perfused with 39°C water to maintain the blood at body temperature (T[subb]). Blood was rapidly cooled by switching the intake of the heat exchanger to a water bath set at 29°C.

**Aerosol administration of hypertonic NaCl and histamine**

Concentrations and durations of aerosol challenges were originally determined through trial and error. Either 14.4% NaCl (4400 mOsm·kg⁻¹), which is similar to that used in human studies [9, 11], or histamine (30 or 50 μg·ml⁻¹) was delivered through the bronchoscope to the obstructed lung segment in the form of aerosols generated by a ultrasonic nebulizer (DeVilbiss Ultra Neb 100). The dual-lumen catheter was temporarily removed from the bronchoscope and the aerosol was delivered through the suction port using 5% CO₂ in air flowing at 200 ml·min⁻¹ for either a 60 s period in the case of hypertonic saline, or for 15 s in the case of histamine.

**Statistical analysis**

P[suba] data were analysed using repeated measures ANOVA and Duncan’s multiple range test. Pre- and post-treatment MAP, mean pulmonary artery pressure (MP[suba]), HR, and T[subb] were compared using paired t-tests. All values represent means±SE. Note that all experiments were of the paired design, and the statistical analysis focused on with-in animal variation. However, the error bars in our figures depict between animal variation. Statistical significance was judged at p<0.05 in all cases.

**Experimental protocols**

Two consecutive challenges were performed in the same wedged segment of the LLL in 22 animals. P[suba] and T[subb] were continuously recorded throughout the experiment. After establishing a stable baseline, the LLL was first challenged at normal T[subb] (~39°C). Approximately 15 min post challenge, the LLL was cooled and a second challenge, identical to the first, was repeated after P[suba] returned to within 10% of the original baseline. Thus, we compared P[suba] in the same wedged segment before, during and after challenge with identical stimuli when T[subb] was maintained at either 39°C or 29°C. An airway wall temperature of 29°C represents the lower limit of temperature change typically recorded during dry air challenge when using this technique [14]. The order in which two challenges were performed was alternated and data were averaged in those experiments in which a challenge was successfully repeated more than once. Several experiments were attempted in each animal. Experiments were done in which the lung periphery was repeatedly exposed to 1) either 1500 or 2000 ml·min⁻¹ dry airflow (5% CO₂ in air) for 2 min, 2) 14.4% NaCl aerosol for 60 s, 3) hypocapnia (200 ml·min⁻¹ compressed air) for 2 min, and 4) 30 or 50 μg·ml⁻¹ histamine aerosol for 15 s. The sensitivity of the animal determined the rate of airflow and the concentration of histamine used in each experiment.
Results

Effect of sublobar cooling on response to dry air challenge

When $T_{na}$ was maintained at $38.1\pm0.4°C$, $P_b$ increased 43±11% (n=6) 5 min after challenge with dry air. When $T_{na}$ was maintained at $29.1\pm0.5°C$, $P_b$ increased only 17±6% 5 min after dry air challenge, and responses were significantly attenuated (p<0.01) throughout the 15 min post-challenge period when compared to values recorded at normal $T_{na}$ (fig. 1). Neither HR (183±10 vs 173±12 b/min, p=0.203), MAP (93±4 vs 90±6 mmHg, p=0.667), $MP_a$ (17±2 vs 19±4 cmH.O, p=0.559), nor $T_b$ (37.0±0.6 vs 36.8±0.6°C, p=0.604) recorded just prior to either challenge differed significantly.

Effect of sublobar cooling on responses to aerosolized hypertonic NaCl

At normal temperatures ($T_{na}=38.4±0.26°C$), a 60 s exposure to 14.4% NaCl aerosol produced a 29±6% (n=7) increase in $P_b$ 30 s post challenge. When the LLL was cold ($T_{na}=29.8±0.23°C$), $P_b$ increased only 15±4% 30 s after challenge, and was significantly attenuated (p<0.01) throughout the 15 min post challenge period when compared to $P_b$'s recorded at normal $T_{na}$ (fig. 2). Neither HR (177±3 vs 179±7, p=0.766), MAP (84±6 vs 88±6, p=0.534), $MP_a$ (18±2 vs 18±2, p=0.966), nor $T_b$ (36.8±0.4 vs 36.2±0.4, p=0.101) were significantly different directly preceding warm and cold challenges, respectively.

Effect of sublobar cooling on response to hypocapnia

When warm ($T_{na}=38.3±0.31°C$), a 2 min exposure to hypocapnia produced a 55±13% (n=6) increase in $P_b$ 30 s after challenge. In contrast, when cold ($T_{na}=30.3±0.39°C$), $P_b$ increased only 23±9% 30 s post challenge and was significantly attenuated 30 s and 2 min post challenge when compared to hypocapnia-induced constriction when warm (fig. 3). Neither HR (164±6 vs 166±11, p=0.825), MAP (107±9 vs 102±7, p=0.139), $MP_a$ (16±2 vs 16±2, p=0.984), nor $T_b$ (36.8±0.4 vs 36.6±0.4, p=0.433) were significantly different before warm and cold challenges, respectively.

Effect of sublobar cooling on responses to aerosolized histamine

When warm ($T_{na}=37.8±0.34°C$), a 15 s exposure to histamine aerosol produced a 54±13% (n=8) increase in $P_b$ 30 s after challenge (fig. 4). When cold ($T_{na}=29.7±0.48°C$), $P_b$ increased 51±18% 30 s post challenge and was not significantly different at 30 s, 2 min, and 10 min post challenge when compared to histamine-induced constriction when warm. In fact, $P_b$ 5 min after cold histamine challenge was significantly
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Fig. 3. - The effect of cooling on \( P_b \) before, during and after two consecutive 2 min hypocapnic challenges in the same sublobar segment. The vertical bar indicates the time of hypocapnic challenge. (A) depicts raw data (n=6), (B) is the above data expressed as % increase above baseline.

Fig. 4. - The effect of cooling on \( P_b \) before, during and after two consecutive 15 s aerosol challenges with histamine (30 or 50 \( \mu \)g·m\(^{-3} \)) in the same sublobar segment. Vertical bar indicates time of aerosol delivery. (A) depicts raw data (n=8), (B) is the above data expressed as % increase above baseline.

Discussion

Cooling the LLL before, during and for 15 min after a 2 min dry air challenge significantly attenuates AIB in the canine lung periphery when compared to measurements recorded in the same sublobar segment when warm (fig. 1). We previously showed that cooling during and for 15 min following dry air challenge virtually abolished AIB in our animal model [5]. Similar data in humans demonstrated that prolonged airway cooling during the recovery period after hyperventilation attenuated AIB [1]. In addition, asthmatic individuals recovering from AIB breathing warm dry air, which would enhance airway cooling relative to recovery while breathing warm humid air, exhibited less airway obstruction [15]. These data are all consistent with the hypothesis that airway cooling down regulates responses to a dry air stimulus.

Marked differences exist in the time-course of responses produced by dry air (fig. 1) and hypertonic aerosol challenge (fig. 2). Responses of canine peripheral airways to dry airflow rise slowly to a maximum 2–5 min after challenge, whereas HIB peaks immediately after aerosol exposure [13]. SMITH and ANDERSON [10] noted similar differences in subjects with asthma, and although unemphasized, a number of studies demonstrate that hypertonic-induced responses develop more rapidly than either hyperventilation or exercise induced asthma [4, 11, 16]. Unlike AIB, aerosol-induced responses occur in the absence of airway cooling, and would be unopposed by an inhibitory stimulus. This cold-associated inhibition may account for the slow onset of constriction that characterizes AIB in our canine model and in human asthmatics. In addition, the absence of inhibition may explain why HIB is not delayed as is AIB, but occurs immediately after exposure [17]. Thus, if drying initiates airway constriction via changes in airway fluid osmolality, then responses to hyperosmotic aerosol and dry air challenges should be similarly affected by airway cooling. The fact that peripheral airway cooling significantly reduces AIB (fig. 1) and HIB (fig. 2) is consistent with the idea that these two agonists operate via similar regulatory pathways.

We previously suggested that airway cooling, via metabolic down regulation, may reduce mediator release and activity, receptor function, cell and tissue responsiveness, and may even stimulate the release, or enhance the production or efficacy of a relaxing factor [6]. If the cold-associated reduction in airway smooth muscle responsiveness observed in this and previous work [5] merely reflects the temperature coefficient \( (Q_{10}) \) of the muscle, or results from the release of an unidentified mediator, the magnitude of change in smooth muscle contractility would be far less than that recorded when warmth is restored. However, MAP (162 ± 2 vs 16 ± 1, \( p=0.803 \)) and HR (172 ± 11 vs 167 ± 11, \( p=0.673 \)) were not significantly different before warm and cold challenges, respectively. However, MAP (102 ± 11 vs 86 ± 10, \( p=0.026 \)) and \( T_b \) (37.9 ± 0.3 vs 37.3 ± 0.2, \( p=0.042 \)) differed significantly before warm and cold challenges, respectively.
relaxant factor, then cooling should indiscriminately reduce responses to all other nonspecific bronchoactive agents. To test these hypotheses, we examined peripheral lung responses to two other nonspecific bronchoactive agents: hypocapnia and aerosolized histamine.

Hypocapnia produces a prompt and marked constrictor response in the canine lung periphery [18, 19]. Although atropine, chlorpheniramine maleate, and indomethacin do not diminish this response, isoproterenol and nifedipine significantly reduce it by 75 and 80%, respectively [18]. In this study, cooling consistently reduced hypocapnia-induced bronchoconstriction (HC-IB) 70% (fig. 3). The mechanism of HC-IB remains unknown, although Kolb et al. [18] speculated that intracellular alkalosis triggers an increase in intracellular calcium via voltage-sensitive channels and results in bronchoconstriction. However, because cooling increases intracellular pH [20] but decreases HC-IB, HC-IB is probably not the result of intracellular alkalosis. Alkalosis does not in itself affect canine tracheal smooth muscle contractile performance [21]. Thus, it is unlikely that alkalosis plays a role in the cold-associated inhibition of either AIB, HIB, or HC-IB.

Airway cooling does not effect histamine-induced bronchoconstriction (His-IB) in our canine model (fig. 4). While HR remained unchanged, MAP for this series of experiments decreased during cooling, and it is possible that bronchial blood flow decreased during this period. However, previous studies demonstrate that bronchial blood flow does not affect the magnitude of response to a histamine challenge [22]. In addition, other experiments in which MAPs were unchanged by cooling confirm this cold-independent response to histamine [17]. Thus, our peripheral lung data suggest that cooling does not indiscriminately reduce mediator release and activity, cell and tissue responsiveness, mediator metabolism, and receptor activity, and thus account for the observed stimulus specific modulation of airway reactivity to dry air, hypertonic NaCl, and hypocapnia.

A number of in vitro studies have examined airway smooth muscle responses to cooling, and may be relevant to AIB. In dogs, responses of canine trachea to electrical field stimulation decreased with decreasing temperature [23]. In human bronchi, cooling reduced baseline tension and inhibited responses to histamine, but increased carbachol-induced contraction [24]. More recently, cooling was reported to relax human airways and attenuated responses to methacholine, histamine and leukotriene C4 [25]. Although inconsistencies exist among the various studies, overall, these in vitro data support the hypothesis that cooling reduces airway responsiveness.

Dry air-induced constriction in the canine lung periphery was previously characterized and described as a model of exercise-induced asthma [7]. The appropriateness and relevance of an animal model depends solely on its ability to mimic the human condition, and the greater the number of similarities that exist between the model and the mimicked process, the better the model. Our canine model can be faulted on three counts: 1) the dog pants, which makes it an unlikely model for hyperpnea-induced bronchoconstriction, 2) unlike human airways, the peripheral airways in our canine model are exposed to unidirectional airflow, and 3) our dewormed conditioned mongrel dogs exhibit no evidence of whole lung hyperreactivity. However, in spite of these differences, the peripheral airways themselves respond to drying in a fashion that is strikingly similar to that reported in human subjects. First, not only is the time course of the response similar to that in man, but responses to variations in stimulus strength and duration are also analogous [7]. Second, the degree of airway cooling is correlated with the magnitude of this response [14]. Third, epithelial cell damage and mediator release is associated with AIB in both asthmatic humans and post challenge dogs [6, 26]. Fourth, humidified air [7, 14], β-agonists [27], methylxanthines [28], and muscarinic receptor antagonists [14] can reduce or abolish AIB. In addition, the attenuation of AIB via the inhibition of cyclooxygenase that was first described in dogs [26], has been recently confirmed in asthmatic humans [29]. Fifth, airway cooling attenuates AIB in dogs [5] and asthmatic humans [1] alike. Sixth, as with humans, a positive correlation between HIB and AIB has been demonstrated in this canine model [4, 13]. Finally, late phase airway obstruction analogous to that reported in asthmatic subjects 3 to 13 h after exercise also occurs in the lung periphery of dogs [30]. Thus, every aspect of AIB examined in the canine lung periphery mimics exercise- or hyperventilation-induced asthma in humans, making this model a useful tool in the interpretation of human data and the testing of alternative hypotheses concerning the pathogenesis of AIB.

As we pointed out in an earlier paper [5], the reduction of an osmotic stimulus in itself could account for the attenuation of AIB seen during sublubar cooling. In those experiments the airway wall temperature (Taw) at the end of a 2 min dry air challenge was 32.4±0.7°C (n=7). If, in our present study, the dry air entering the wedged segment was fully saturated at 32.4°C, then water content of the air would increase to ~34 mg·l-1. Based on our earlier work, water content at the end of the cold challenge (Taw=28.8±0.62°C) would be ~28 mg·l-1 and water loss during a 2 min warm and cold challenge (1500 ml·min-1) would be ~95 and 79 mg, respectively. We do not believe that an estimated 16 mg difference in water loss in itself can account for the 66% reduction in AIB reported in this study. Thus, although a reduction in stimulus strength may account for some of the cold-associated reduction in AIB seen in fig. 1, we believe that cooling acts to attenuate AIB at a point in the metabolic pathway downstream from the initiating stimulus. The fact that cooling inhibits hypertonic saline-induced responses (fig. 2) is consistent with this idea in that cooling can alter mediator release from osmoregulating cells [31]. Since cooling per se is clearly not the stimulus to AIB in the canine lung periphery [5], attenuated responses in conjunction with cold associated reductions in the strength of the osmotic stimulus, i.e., water loss, strongly support the airway drying hypothesis.
The point is, cooling reduces the response, it does not enhance it. The fact that the trachea of asthmatic subjects rewarms more rapidly than normal individuals [2] suggests that during the post-exercise recovery period, asthmatics lose more water than their normal counterparts. This enhanced post-exercise rewarmin suggests a cold associated inhibitory process may be impaired in asthmatic individuals.

In conclusion, cooling reduces dry air-, hypertonic aerosol-, and hypocapnia-induced constriction of canine peripheral airways. In contrast, cooling does not alter histamine-induced constriction, suggesting that neither histamine receptor, histamine-sensitive cell, nor airway smooth muscle functions are affected by cooling. Thus, cold associated down regulation of airway reactivity in the canine lung periphery is stimulus dependent. We speculate that dry air-, hypertonic aerosol-, and hypocapnia-induced responses are modulated by airway cooling via a cold associated inhibition of stimulus specific neuronal activity or mediator release during challenge. In addition, the cold associated reduction in AIB may be partially related to a diminution in stimulus strength. Finally, cold-induced inhibition of dry air- and hypertonic aerosol-induced constriction is consistent with the hypotheses that 1) alterations in the pulmonary osmotic milieu initiates AIB [3, 4], and 2) airway cooling antagonizes the bronchoconstriction that results from airway drying [5].

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References


RÉSUMÉ: Nous avons utilisé une technique de bronchoscope bloqué, en conjonction avec une préparation de lobe inférieur gauche perfusé et isolé in situ, chez de chiens anesthésiés, pour examiner la modulation de réponse pulmonaires périphérique associées au froid, à l'égard du courant gazeux sec, de l'hypocapnie, et d'aérosols d'histamine et de chlorure sodique hypertonique. Dans cette préparation, la température des parois des voies aériennes a été abaissée rapidement, par diminution de la température du sang perfusant le segment sous-lobaire bloqué. Le refroidissement atténué de façon significative les réponses à l'air sec, à l'aérosol de chlorure sodique hypertonique, et aux provocations hypocapniques. Au contraire, le refroidissement n'affecte pas les réponse de la périphérie pulmonaire à l'égard de l'aérosol d'histamine. Donc, le refroidissement en lui-même n'inhibe pas la réponse du muscle lisse. Nous concluons que, selon le stimulus en cause, le refroidissement peut moduler la réactivité de la voie aérienne.

Nous supposons que le refroidissement atténue les bronchospasmes dus à l'hypocapnie, aux aérosols hypertoniques et à l'air sec, par une réduction de leur activité neuronale ou de la production et de la libération de médiateurs sous l'effet du froid.