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Studies of Mediator Involvement in Cooling-Induced Alterations of Guinea Pig Tracheal Smooth Muscle Contractility (42640)

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Abstract. Heat loss from airway smooth muscle is a potent stimulus which causes substantial, but poorly understood, alterations in muscle tension. This study considered the involvement of endogenous mediators in cooling-induced tension changes in incubated guinea pig trachea. Smooth muscle tension was monitored in tracheal cylinders which were carefully cooled from 37 to 30°C in the presence or absence of various inotropic mediators. In our study, cooling alone, at a rate of 1°C/min, was associated with an average loss of smooth muscle tension of 88.2 mg. Cooling tracheal tissue that had been previously exposed to 3×10^{-6} *M* histamine, however, caused an additional increase in tracheal tension of 133 mg, over and above that caused by histamine alone. In the presence of 10^{-5} *M* prostaglandin $F_{2\alpha}$, or 10^{-5} *M* thromboxane B_2, cooling was associated with respective losses of smooth muscle tension of 211.4 and 211.2 mg, as compared to the tension associated with these mediators when they were used alone under control conditions. When the speed of tracheal cooling was increased to 40°C/min, there was a slight increase in tension for 20 sec followed by a pronounced and sustained relaxation. The mechanisms involved in the response of airway smooth muscle to cooling are complex. The results of our study, however, suggest that mediators may play a role in the cooling-induced alterations of airway smooth muscle tension.

An accelerated rate of heat loss from the respiratory tract during exercise is a stimulus for severe bronchoconstriction in susceptible humans and is responsible, in part, for a condition known as exercise-induced asthma (1). The mechanism by which localized cooling causes airway smooth muscle to contract is poorly understood. The pathways involved are essentially unknown but may involve vagal efferents or released mediators which may act directly or indirectly to induce the contractile event (2, 3).

The general concept of mediator release, as related to exercise-induced asthma, is supported by several observations. For example, airway cooling during exercise is a transient event, whereas airflow limitation is progressive and lasts much longer (4). Also, the symptoms of exercise-induced asthma are muted by pharmacological agents which inhibit mediator release from mast cells lying superficially in the bronchial mucosa (1, 5). The depletion of these mediators is thought to be responsible for the "refractory" period which often follows an episode of exercise (6).

The original aim of this study was to characterize the mechanisms involved in the process by which cooling causes an increase in airway smooth muscle tension. However, when using the isolated guinea pig trachea as a model to study this phenomenon, we discovered that cooling under control conditions was associated with a decrease in smooth muscle tension—not an increase as was expected. Only in the presence of histamine were we able to demonstrate a cooling-induced contractile event. This paper, therefore, evaluates the influence of histamine and other endogenous mediators in cooling-induced changes of airway smooth muscle tension. Preliminary results of this study have been previously presented at national meetings (7–10).

Materials and Methods. *Tissue preparation.* Male Hartley guinea pigs weighing 350–500 g were killed by a sharp blow to the head. After midsternal thoracotomy, the tracheas were excised and immersed in 37°C oxygenated physiological medium containing (m*M*) NaCl, 118.4; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 8.9; $CaCl_2$, 2.5; and glucose, 5.6. After excess tissue was removed, the tracheas were divided into eight cylindrical segments, each 4 mm in length. After the segments were randomized to min-

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imize regional tracheal variability, they were mounted on opposing L-shaped 30-gauge needles with considerable care being taken to preserve the tracheal endothelium (11). Each tracheal preparation was suspended in an individual water-jacketed incubation chamber containing 20 ml of the physiological medium which was aerated continuously with a mixture of O_2 and CO_2 at 37°C and a pH of 7.35. One of the needles was anchored to the bottom of the bath, while the other was attached with 5-O silk to a Statham VC3 force-displacement transducer interfaced with a UL-5 microscale attachment. A preload stress was applied to the tracheal rings by moving the needles apart. Signals from the transducers were amplified and recorded on a physiological recorder (Beckman dynograph) as grams of force. The tissue was allowed to equilibrate for 90 min under a resting tension of 2 g, a tension found to achieve the best tissue stability and responsiveness as determined by the method of Stephens *et al.* (12).

Mediators. Following the 90-min equilibration period, each ring segment was exposed, at 37°C, to one of a group of endogenous mediators known to change smooth muscle tension. These mediators included histamine, prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), or thromboxane B_2 (TXB_2). These mediators are not an all-inclusive group of inotropic agents; they are those whose contractile characteristics have been reasonably well defined. Each agent was added to the incubating bath 15 min before the initiation of cooling in a concentration known to cause a maximal muscle response (previously determined in dose–response experiments). The concentration of each agent necessary to elicit a maximal response and the resulting tracheal tension are as follows: 3×10^{-6} *M* histamine caused a gain of 182.6 ± 12.2 g/g wet wt, 10^{-5} *M* TXB_2 caused a gain of 23.1 ± 1.7 g/g wet wt, 10^{-5} *M* $PGF_{2\alpha}$ caused a gain of only 1.3 ± 0.2 g/g wet wt, and 7×10^{-6} *M* PGE_2 caused a loss of 5.3 ± 0.8 g/g wet wt. In some experiments the H_1-blocking agent diphenhydramine was added to the bath 15 min prior to histamine at a concentration of 10^{-5} *M*, a level sufficient to negate the constrictive influence of the agonist.

Smooth muscle reactivity to external stimuli depends, in part, upon the tension sustained by it (12). Prior to the induction of cooling, therefore, the tracheal tension was readjusted to 2 g. This adjustment nullified the variations in smooth muscle tension induced by the various mediators. Temperature, therefore, became the independent experimental variable and smooth muscle tension was the dependent variable. Without temperature change, the tension of the tracheal rings remained constant. After tension readjustment, the tracheal rings were nonresponsive to additional increments of mediators.

Cooling. The influence of cooling on tension development was evaluated by carefully lowering the temperature of the incubation baths within which the tracheal cylinders were immersed. The cooling occurred in the 7°C range between 37 and 30°C and was monitored with temperature probes (Yellow Springs, OH) immersed in the incubation baths. The signals from the temperature probes and the force-displacement transducers were recorded simultaneously and could be accurately related to one another on the same time axis. The tracheal rings were cooled at two different rates, at 1 and at 40°C/min. The slower rate of cooling was similar to that recorded by Deal *et al.* (13) in the esophageal tissue adjacent to the trachea in human subjects breathing −17.8°C air. Lowering the temperature of the tissue baths at 1°C/min was achieved by a gradual cooling of the fluid in the incubation chamber's water jacket using a Haake cooling pump. Cooling at 40°C/min, on the other hand, was accomplished by a bulk displacement of the incubation fluid with cooler fluid, so that the final bath temperature was 30°C. In the latter situation, a bulk displacement of the incubation fluid without cooling did not, in itself, change the contractile tone.

Controls. Controls were used in experiments in which tracheal rings were exposed to tissue mediators and to alterations in temperature. Mediator-induced changes in smooth muscle tension were related to tension measurements made in tracheal rings incubated under the same conditions but without the mediators. Cooling-induced tension changes in the presence of mediators

were compared to tension changes measured in control tracheal rings incubated under the same conditions but held at a constant 37°C. Stock solutions of prostaglandins and thromboxane were prepared in dimethyl sulfoxide. To negate the effects of DMSO on the tracheal tissue, equivalent amounts of this solvent were also added to control baths.

pH. The pH of the incubation baths was continuously monitored throughout the experimental procedure. A servomechanism on the pH meter was designed to correct alterations in solution pH. Any deviation from pH 7.35 activated an adjustable regulator valve on the CO_2 cylinder causing more or less CO_2 to enter the O_2–CO_2 aerating gas mixture. In this manner, the usual temperature-induced alterations in solution pH were avoided. At 37°C the aerating gas mixture was approximately 95% O_2 and 5% CO_2. Cooling at 40°C per min, however, was accomplished by a bulk displacement of the incubation fluid with cooler fluid whose temperature was 30°C and whose pH was already adjusted to 7.35.

Statistics. The results of all experiments were pooled and analyzed statistically with *n* referring to the number of animals used. Means and standard errors of the means (SEM) were determined. Rates of tension change, as related to degrees of cooling, were calculated by a least-squares fit of the data. The correlation coefficient of this relationship was also calculated and is noted in the figure legends.

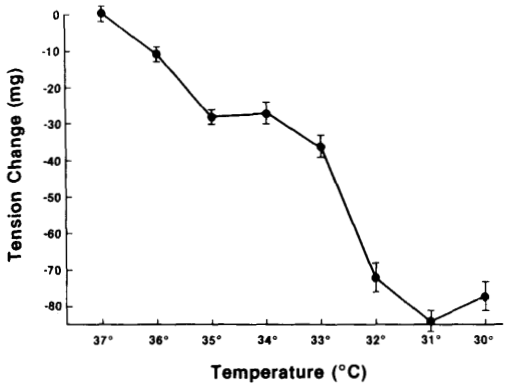

FIG. 1. The influence of slow cooling on tension change in isolated guinea pig tracheal cylinders. The rate of cooling was 1°C/min. The data are expressed as milligrams of tension with temperature as the independent variable and have a correlation coefficient of 0.95. The points are mean values of 57 experiments with the standard error of the means indicated.

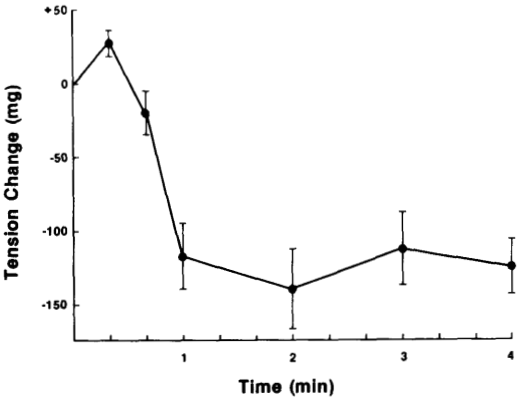

FIG. 2. The influence of rapid cooling on tension change in isolated guinea pig tracheal cylinders. The rate of cooling was 40°C/min. The data are expressed as milligrams of tension with time as the independent variable. The points are mean values of 20 experiments with the standard error of the means indicated.

Results. *Control studies.* Figure 1 shows the result of cooling the incubated tissue through the 7° temperature range of 37 to 30°C at a rate of 1°C/min. Under these conditions, cooling was associated with tracheal smooth muscle relaxation of 88.2 mg at an average rate of 12.6 mg per 1°C. Figure 2 shows the tension change of tracheal segments cooled through the same 7° temperature range but at the rate of 40°C/min. Rapid cooling was associated with a slight increase in tension for the first 20 sec, followed by a pronounced and sustained relaxation. The entire tension change needed 2 to 3 min to happen, a time much longer than the brief cooling perturbation. For this reason, the figure is plotted with time as the independent variable. Rewarming the tissue to 37°C caused tracheal tension to return to precooled levels.

Histamine–antihistamine studies. In the presence of 3×10^{-6} *M* histamine, cooling at 1°C/min caused a progressive increase in tracheal tension over and above that developed in the presence of histamine alone. Under these conditions, cooling caused an increase in tension of 133 mg, developed at the rate of 19 mg per 1°C (Fig. 3). When 10^{-5}

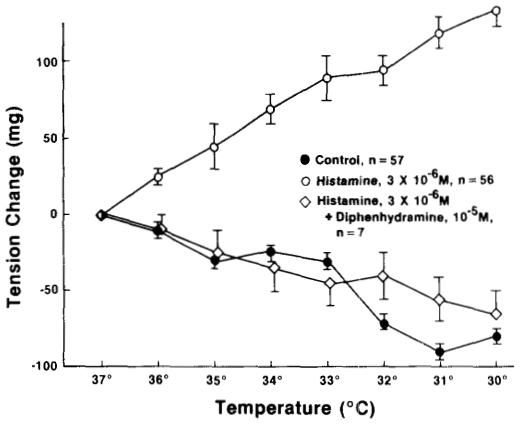

FIG. 3. The influence of slow cooling on tension change in isolated guinea pig tracheal cylinders exposed to histamine ± diphenhydramine. The rate of cooling was 1°C/min. The data are expressed as milligrams of tension with temperature as the independent variable and have correlation coefficients of 0.95 for control conditions, 0.97 in the presence of histamine, and 0.70 in the presence of histamine + diphenhydramine. The points are mean values of *n* experiments, each with the standard error of the means indicated.

M diphenhydramine was added to the bath, however, cooling in the presence of histamine was associated with 65.8 mg of tracheal relaxation at an average rate of 9.4 mg per 1°C. This rate of relaxation was only slightly less than that observed under control conditions (Fig. 1). Cooling-induced alterations of tracheal contractility was also studied in the presence of lesser concentrations of histamine. The effect of cooling became more pronounced in the presence of greater histamine concentrations with cooling-associated "constrictions" occurring in the presence of histamine concentrations greater than 10^{-8} *M*. Figure 3 displays the more pronounced data obtained using histamine concentrations of 3×10^{-6} *M*. In all cases, rewarming to 37°C caused tension to return to precooled levels.

Prostaglandin studies. As noted in Fig. 4, cooling tracheal rings at 1°C/min in the presence of 7×10^{-6} *M* PGE_2 was related to a slight, albeit nonstatistically significant, drop in tracheal tension over the entire 7° cooling range. Cooling, in the presence of either 10^{-5} *M* $PGF_{2\alpha}$ or 7×10^{-6} *M* TXB_2, was associated with a more pronounced "overall" loss of smooth muscle tension. For TXB_2, however, the tension response was biphasic with contraction occurring from 37 to 34°C and relaxation occurring from 34 to 30°C. The absolute changes in tension noted over the entire temperature range studied are as follows: PGE_2 was associated with a loss of 1.3 mg/°C and $PGF_{2\alpha}$ was associated with a loss of 30.2 mg/°C. For TXB_2 there was a 52.8 mg/°C loss of tension for the 4° temperature drop from 34 to 30°C. Rewarming to 37°C caused tension to return to precooled levels.

Discussion. The concept of exercise-induced asthma is based, in part, upon the finding that the airway cooling associated with exercise causes bronchoconstriction in susceptible people (1). The results of our study, however, show that such airway constriction probably occurs only in the presence of certain mediators, as for example, histamine. Without any added mediators, we found that cooling at a rate similar to that recorded in the esophageal tissue adjacent to the trachea in humans breathing −17.8°C air (13), caused sustained smooth muscle relaxation (Fig. 1). Such cooling-induced relax-

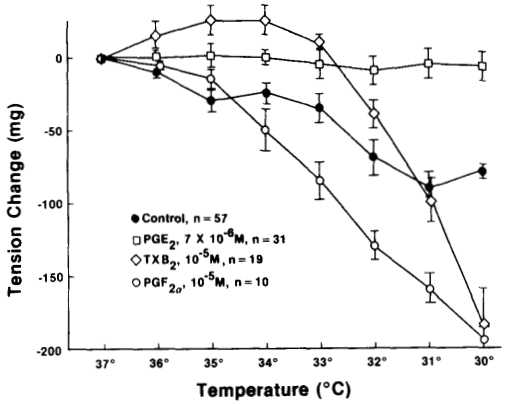

FIG. 4. The influence of slow cooling on tension change in isolated guinea pig tracheal cylinders exposed to various arachidonic acid metabolites. The rate of cooling was 1°C/min. The data are expressed as milligrams of tension with temperature as the independent variable and have correlation coefficients of 0.95 for control conditions, 0.84 in the presence of PGE_2, 0.97 in the presence of TXB_2 (34 to 30°C), and 0.98 in the presence of $PGF_{2\alpha}$. The points are mean values of *n* experiments, each with the standard error of the means indicated.

ation may be the result of an "absence" of constricting mediators or, on the other hand, of the "presence" of locally released relaxing mediators. The delayed contractile response which occurs after rapid cooling (Fig. 2) could also implicate mediator involvement in cooling-induced alterations of contractility.

Available evidence suggests that the tension responses of airway smooth muscle to cooling may be mediator dependent (2–6). The identity of these endogenous factors, however, is not known with certainty. We found that tracheal smooth muscle contracted in response to cooling when exposed to histamine, but relaxed in the absence of histamine and when histamine was antagonized by the H_1 blocker, diphenhydramine (Fig. 3). Such findings, if extrapolated to *in vivo* situations, might show a potential role for histamine in cooling-induced airway constriction. Indeed, several *in vivo* studies have noted small increases in plasma histamine levels after exercise (14, 15) and have found that airway sensitivity to histamine is increased after exercise (16). Antihistamines, delivered either as aerosols (17) or by intramuscular injection (18), have been shown to be effective in the prevention of exercise-induced asthma. The role of histamine in exercise-induced asthma, however, is not universally accepted. Park *et al.* (19) found that the early increase in tension, following cooling at a rate of about 4°C/min, was insensitive to diphenhydramine and concluded that histamine probably was not involved in exercise-induced asthma. These investigators, however, performed their incubation studies without an exogenous source of histamine, in which case the absence of a response to diphenhydramine would only show that histamine was not present in the incubated tissue being studied. Caution must, of course, be exercised when extrapolating the histamine data of our study to *in vivo* situations. In our study, the tracheal smooth muscle are exposed to relatively high concentrations of histamine before cooling. In EIA, however, the sequence of events is probably reversed with cooling preceding the release of histamine.

Souhrada *et al.* (20) have recently presented information which shows that metabolites of arachidonic acid may also be involved in cooling-induced smooth muscle tension changes. Using tracheal segments, these investigators found that aspirin potentiated the early, contractile aspect of a cooling-induced biphasic response. Park *et al.* (19), however, did not observe a potentiating effect in the early contractile response when using indomethacin. Our studies appear to support those of Souhrada *et al.* (20), in that we found that arachidonic acid metabolites influenced the cooling-induced tracheal relaxation response (Fig. 4). The effects of PGE_2 and $PGF_{2\alpha}$, like those of cooling under control conditions, are surprising and interesting. In *in vivo* situations, $PGF_{2\alpha}$ has been reported to exhibit histamine-like effects, because it causes bronchoconstrictions in asthmatics (21). Alternatively, PGE_2, presumably by increasing cAMP, is thought to promote airway smooth muscle relaxation (22). In the presence of cooling in our study, however, PGE_2 appears to block the cooling-induced relaxation noted in the absence of mediators, while $PGF_{2\alpha}$ appears to accelerate it (Fig. 4). $PGF_{2\alpha}$, in fact, appears to double the relaxation when compared to tension changes seen with cooling alone. In the presence of $PGF_{2\alpha}$, slow cooling causes approximately the same degree of relaxation as that noted with rapid cooling (Fig. 2). Further, in the presence of TXB_2, the slow cooling-induced tension response was biphasic and resembled the tension changes noted for rapid cooling.

The humidity of inspired air has been reported to be important in determining the degree of bronchoconstriction experienced by asthmatics in response to exercise (23). It is hypothesized that osmotic changes related to water loss, instead of heat loss, are the important stimuli for exercise-induced asthma. The results of our studies of isolated tracheal cylinders incubated in physiological solutions of constant tonicity (Figs. 1 and 2), show that cooling alone is enough stimulus to trigger smooth muscle tension changes *in vitro*.

It has been reported that cooling causes airway smooth muscle to become more sensitive to various agonists. For example, Downing *et al.* (24) found the tracheal tissues of guinea pigs at 30°C to be more sensitive to

isoproterenol and histamine than those being incubated at 40°C. Wagner *et al.* (25) also reported a cooling-enhanced sensitivity in guinea pig tracheal rings to vasoactive catecholamines. Explanations of this poorly understood concept of cooling-induced supersensitivity include the conversion of β- to α-adrenergic receptors (26) and the partial inhibition of the sarcoplasmic calcium pump, resulting in increased cytoplasmic calcium levels (27). Whatever mechanisms may be involved, our study shows that they are reversible. Rewarming the tissue to 37°C causes the tension to return to precooled levels.

Many, often conflicting hypotheses have been advanced to explain the pathways linking airway cooling to bronchoconstriction. Some confusion undoubtedly stems from a lack of clear, definitive, *in vivo* data. For example, no study has yet been able to accurately make direct measurements of the rate, or the degree, of airway cooling encountered in situations leading to exercise-induced asthma, albeit, Deal *et al.* (13) recorded temperatures in esophageal tissue adjacent to the trachea in human subjects. The rate of cooling, as our data and those of others (19, 28, 29) show, is an important factor to consider when studying the tension response of airway smooth muscle to cooling. The degree of airway cooling undoubtedly varies, depending upon the site within the respiratory tract considered. Our findings concerning the involvement of endogenous tissue mediators are, undoubtedly, far from complete. Besides histamine and the arachidonic acid metabolites considered in this study, other arachidonic acid metabolites (via the cyclooxygenase or lipoxygenase pathways) or neurotransmitters, etc., may also be involved. Based on our results, however, it seems clear that cooling-induced bronchoconstriction is mediator dependent.

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1. McFadden ER, Ingram RH. Exercise-induced airway obstruction. Annu Rev Physiol **45**;453–463, 1983.
2. Findlay SR, Lichtenstein LM. Basophil "releasability" in patients with asthma. Amer Rev Respir Dis **122**:53–59, 1980.
3. Lee TH, Nagakuro T, Papageorgiou N, Cromwell O, Iikura Y, Kay AB. Mediators in exercise-induced asthma. J Allergy Clin Immunol **73**: 634–639, 1984.
4. McFadden ER, Pichurko BM, Soter NA, Ringel EW, Mefford IM. Respiratory heat exchange and the asthmatic response. In: Kay AB, Austen KF, Lichtenstein LM, Eds. Asthma: Physiology, Immunopharmacology, and Treatment. London, Academic Press, pp 315–326, 1984.
5. Anderson SD, Rozea PJ, Dolton R, Lindsay DA. Inhaled and oral bronchodilator therapy in exercise-induced asthma. Aust N Z J Med **5**:544–550, 1975.
6. Edmunds AT, Tooley M, Godfrey S. The refractory period after exercise-induced asthma: Its duration and relation to the severity of exercise. Amer Rev Respir Dis **117**:247–254, 1978.
7. Chaudhary BA, Kolbeck RC, Speir WA. Effect of thermal change on tracheal smooth muscle response to PGE_2 and histamine. Clin Res **29**:844, 1982.
8. Chaudhary BA, Kolbeck RC, Speir WA. Thermal changes and tracheal smooth muscle tone: Is increased reactivity, mediator-dependent? Amer Rev Respir Dis **123**:80, 1981.
9. Kolbeck RC, Chaudhary BA, Speir WA. Effects of PGE_2, $PGF_{2\alpha}$, TXB_2, and histamine on tracheal smooth muscle response to thermal change. Amer Rev Respir Dis **127**:109, 1983.
10. Kolbeck RC, Chaudhary BA, Gill HK, Speir WA. Diphenhydramine and tracheal smooth muscle response to thermal change: An *in vitro* model. Chest **80**:355–356, 1981.
11. Furchgott RF. The role of endothelium in the responses of vascular smooth muscle to drugs. Annu Rev Pharmacol Toxicol **24**:175–197, 1984.
12. Stephens NL, Kroeger E, Menta JA. Force-velocity characteristics of respiratory airway smooth muscle. Amer J Physiol **26**:685–692, 1969.
13. Deal EC, McFadden ER, Ingram RH, Jaeger JJ. Esophageal temperature during exercise in asthmatic and nonasthmatic subjects. J Appl Physiol **46**:484–490, 1979.
14. Anderson SD, Bye PTP, Schoeffel RE, Seale JP, Taylor KM, Ferris L. Arterial plasma histamine levels at rest, during and after exercise in patients with asthma: Effects of terbutaline aerosol. Thorax **36**:259–267, 1981.
15. Shaw R, Durham S, Torzillo P, Schoeffel RE, Anderson SD, Kay AB. Neutrophil chemotactic activity following the inhalation of ultrasonically nebulized water. Aust N Z J Med **13**:541–549, 1983.
16. Hahn AG, Nogrady SG, Tumilty DM, Lawrence SR, Morton AR. The role of histamine depletion in the refractory period after exercise-induced asthma. Thorax **39**:919–923, 1984.

17. Hartley JPR, Nogrady SG. Effect of an inhaled antihistamine on exercise-induced asthma. Thorax **35**:675–679, 1980.
18. Zielinski J, Chodosowska E. Exercise-induced bronchoconstriction in patients with bronchial asthma: Its prevention with an antihistaminic agents. Respiration **34**:32–35, 1977.
19. Park MK, Hayashi S, Robotham JL. Cooling-induced contraction of guinea pig tracheal smooth muscle. Proc Soc Exp Biol Med **177**:283–289, 1984.
20. Souhrada FJ, Presley D, Souhrada M. Mechanisms of the temperature effect on airway smooth muscle. Respir Physiol **53**:225–237, 1983.
21. Hargreave FE, O'Byrne PM, Ramsdale EH. Mediators, airway responsiveness, and asthma. J Allergy Clin Immunol **76**:272–276, 1985.
22. Moore DH, Abel PW, Rinard GA, Puckett AM, Walter JB. Regional differences in relaxation of electric field-stimulated canine airway smooth muscle by verapamil, isoproterenol and prostaglandin E_2. J Pharmacol Exp Ther **238**:76–82, 1986.
23. Anderson SD. Current concepts of exercise-induced asthma. Allergy **38**:289–302, 1983.
24. Downing D, Kolbeck RC, Holl JE, Bransome ED, Speir WA. Temperature dependence of tracheal smooth muscle to isoproterenol. Life Sci **24**:603–608, 1979.
25. Wagner J, Reinhardt D, Schumann HJ. Sensitivity changes of adrenergic beta-receptors of isolated ileum and tracheal preparations induced by alteration of temperature. Arch Int Pharmacodyn **197**:290–300, 1972.
26. Kunos G, Nickerson M. Effects of sympathetic innervation and temperature on the properties of rat heart adrenoceptors. Brit J Pharmacol **59**:603–614, 1977.
27. Reiter M, Stickel FJ. Zur Frage einer therapeutischen Steigerung der Kontraktilitat bei Abkuhlung des Herzens. Klin Wschr **48**:935–938, 1970.
28. De Carle DJ, Szabo AC, Christensen J. Temperature dependence of responses of esophageal smooth muscle to electrical field stimulation. Amer J Physiol **232**:E432–E436, 1977.
29. Sunano S. Low temperature-induced contracture of depolarized smooth muscle and the effects of calcium and multivalent cations. Experientia **37**;1165–1166, 1981.

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