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European Journal of Pharmacology 296 (1996) 291–296

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Cold air-induced bronchoconstriction is mediated by tachykinin and kinin release in guinea pigs

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Received 16 October 1995; accepted 24 October 1995

Abstract

In the present study, we investigated the role of acetylcholine, tachykinins and kinins in the bronchoconstriction induced by cold air inhalation. Cold air was delivered to anaesthetised, artificially ventilated guinea pigs through a tracheal cannula. Inhalation of cold air increased the maximum total pulmonary resistance (R_L) in a time-dependent manner, reaching a maximum after 15 min of exposure. The increase in R_L induced by exposure to cold air for 10 min was not affected by pretreatment with atropine (1.4 μmol/kg, i.v.); it was abolished by the tachykinin NK_2 receptor antagonist, SR 48968 (0.3 μmol/kg, i.v.) and was reduced by 58% by the kinin B_2 receptor antagonist, HOE 140 (0.1 μmol/kg, i.v.) These findings suggest that cold air induces bronchoconstriction in guinea pigs via a cascade that involves the release of kinins and tachykinins.

Keywords: Cold air; Tachykinin; Kinin; Bronchoconstriction; SR 48968; HOE 140; Neurogenic inflammation

1. Introduction

Breathing cold air increases the bronchial responsiveness to inhaled histamine in asthmatic patients (Dosman et al., 1991) and enhances exercise-induced asthma (Strauss et al., 1977). The hypothesis that exposure to cold air may affect bronchial tone is suggested by the observation that asthma-like symptoms and airway hyperresponsiveness are much more common in cross-country skiers (breathing large volumes of cold air) than in the general population and in non-skiers (Larsson et al., 1993).

Previously we reported that plasma extravasation induced by inhalation of cold air in the rat trachea is mediated by tachykinin release from sensory nerves. We also showed that kinin release plays a role in this response (Yoshihara et al., 1995). Cold receptors are present on different types of afferent nerve fibers. Capsaicin, which selectively stimulates tachykinin-containing sensory nerves, can stimulate cold receptors belonging to C- and Aδ-fibers (Seno and Dray, 1993). Tachykinins released from sensory nerve endings in the airways induce a series of inflammatory responses, collectively referred to as neurogenic inflammation (Barnes, 1986; Holzer, 1988; Solway and Leff, 1991; Nadel, 1992). Contraction of the tracheobronchial smooth muscle is one of the effects produced by neurogenic inflammation.

Bronchoconstriction caused by sensory nerve stimulation and mediated by tachykinins in the guinea pig airways is mostly due to activation of tachykinin NK_2 receptors (Maggi et al., 1991; Ballati et al., 1992; Bertrand et al., 1993a, b; Lilly et al., 1994), although tachykinin NK_1 receptors may also play a role (Bertrand et al., 1993a, b). Upon stimulation of sensory nerves, reflexes involving the activation of parasympathetic efferent nerves may also contribute to the bronchoconstriction (Maggi et al., 1991; Ballati et al., 1992).

The aim of the present study was to determine whether exposure to cold air increases total pulmonary resistance (R_L) in anaesthetised, artificially ventilated guinea pigs. Because we found that cold air markedly increased airflow resistance, we explored the mechanism(s) responsible for cold air-induced bronchocon-

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Elsevier Science B.V.
SSDI 0014-2999(95)00719-9

striction. In particular, we examined the contribution of acetylcholine and tachykinins, two major neural bronchoconstrictor systems of the guinea pig airways. We also examined the possible involvement of inflammatory mediators, including histamine, prostanoids and bradykinin, which may be released upon exposure to cold air.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Simonsen Laboratories, Gilroy, CA) weighing 300–350 g at the time of housing, were used in this study. They were kept in a temperature-controlled environment with standard laboratory food and water freely available.

2.2. Exposure to cold air

Guinea pigs were anaesthetised using sodium pentobarbital (45 mg/kg, i.p.; Anthony Product Corp., Arcadia, CA) and artificially ventilated with a tracheal cannula, using a constant-volume ventilator (model 683; Harvard Apparatus Co., South Natick, MA) set at a frequency of 90 breaths/min. The tidal volume was adjusted to maintain normal arterial blood gases as described previously (Dusser et al., 1988). A cannula with a conical tip and an inner diameter of 1 mm was inserted for 1–3 mm between the cricoid cartilage and the first tracheal ring. Cold air was delivered by the administration of air which passed through a coiled tubing immersed in ground dry ice before reaching the cannula (Yoshihara et al., 1995). The temperature of the air recorded at the distal end of the cannula prior to insertion in the trachea was 10°C, while the room temperature was 26°C. To maintain a constant humidification, cold air was inhaled together with a mist of nebulised 0.9% saline (Pulmo-Sonic 25, Devillbiss, Somerset, PA). The nebulised 0.9% saline was mixed with cold air after the air had passed through the coiled tubing immersed in dry ice. The temperature of the tracheal cannula was measured before and after each experimental trial to determine whether the temperature remained constant throughout the study. Saline aerosol was also given to the animals during inhalation of room air.

2.3. Measurement of total pulmonary resistance (R_L)

Airflow was monitored continuously using a pneumotachograph (A. Fleisch, Medical, Richmond, VA) connected to a differential pressure transducer (model DP45; Validyne Engineering Corp., Northridge, CA). A fluid-filled polyethylene catheter was introduced into the oesophagus to measure the oesophageal pressure as an approximation of pleural pressure. Intratracheal pressure was measured using a polyethylene catheter inserted into a short tube connecting the tracheal cannula to the pneumotachograph. The transpulmonary pressure (defined as the pressure difference between the intratracheal and the oesophageal pressures) was measured with a differential pressure transducer (model DP7; Valydine Engineering Corp.). Output signals representing transpulmonary pressure and airflow were amplified (amplifier model CD19; Validyne Engineering Corp.) and recorded on a polygraph recorder (model 1508 B Visicorder; Honeywell, Denver, CO). R_L was calculated as previously described (Dusser et al., 1988). The right jugular vein and the left carotid artery were cannulated to permit administration of drugs and withdrawal of blood samples for arterial blood gas measurements.

2.4. Experimental design

No significant changes in R_L were produced by an intravenous injection of 0.9% NaCl (1 ml/kg) after a stabilization period of 30 min, and the baseline R_L remained stable for at least 2 h during exposure to room air.

Atropine (1.4 μmol/kg, i.v.) was administered 15 min before the exposure to cold air. The effects of tachykinin NK_2 receptor antagonist (SR 48968; 0.03, 0.1 and 0.3 μmol/kg, i.v., 15 min before the exposure to cold air) (Emonds-Alt et al., 1992), the kinin B_2 receptor antagonist (HOE 140; 0.01, 0.05 and 0.1 μmol/kg, i.v., 15 min before the exposure) (Hock et al., 1991), the histamine H_1 receptor antagonist (pyrilamine, 10 mg/kg, i.p., 30 min before the exposure), the H_2 receptor antagonist (cimetidine, 10 mg/kg, i.p., 30 min before the exposure) (Ichinose et al., 1990a), and the cyclooxygenase inhibitor, indomethacin (5 mg/kg, i.v., 30 min before the exposure) (Ichinose et al., 1990b) were examined.

2.5. Drugs

Atropine, indomethacin and pyrilamine were obtained from Sigma Chemical (St. Louis, MO). Cimetidine was a kind gift of Smith Kline Beecham Pharmaceuticals (Philadelphia, PA). SR 48968 {(*S*)-*N*-methyl-*N*-[4-(acetyl-amino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl]benzamide} was kindly provided by Dr. X. Emonds-Alt (Sanofirecherche, Montpellier, France). HOE 140 (D-Arg-[Hyp^3,This^5,D-Tic^7,Oic^8]-bradykinin) was a kind gift of Dr K.J. Wirth (Hoechst AG, Frankfurt, Germany). Drugs were dissolved in 0.9% saline or in dimethyl sulfoxide (Hoe 140 and SR 48968). Further dilutions were made in 0.9% saline.

2.6. Statistical analysis

All data are expressed as means ± S.E.M.. Statistical comparisons were performed using a one way analysis of variance and Dunnett's test or bilateral unpaired Students *t*-tests, when appropriate. In all cases, a P value of less than 0.05 was considered significant.

3. Results

Cold air exposure increased R_L, an effect that correlated well with increases in the duration of exposure (Fig. 1): exposure to cold air for 5 min failed to increase R_L significantly (Fig. 1); an increase in R_L was evident after a 10 min exposure to cold air and reached a maximal bronchoconstrictor response after 15 min exposure (Fig. 1). The increase in R_L induced by inhalation of cold air for 10 min was not significantly affected by pretreatment with atropine (1.4 μmol/kg, i.v.) (Fig. 2b and Fig. 3), but it was abolished by the tachykinin NK_2 receptor antagonist (SR 48968, 0.3 μmol/kg, i.v.) (Fig. 2c and Fig. 4). Administration of either SR 48968 (0.03, 0.1 and 0.3 μmol/kg, i.v.) or the kinin B_2 receptor antagonist, HOE 140 (0.01, 0.05 and 0.1 μmol/kg, i.v.) via the jugular vein produced dose-related inhibition of the peak increase in R_L induced by cold air exposure for 10 min (Fig. 5).

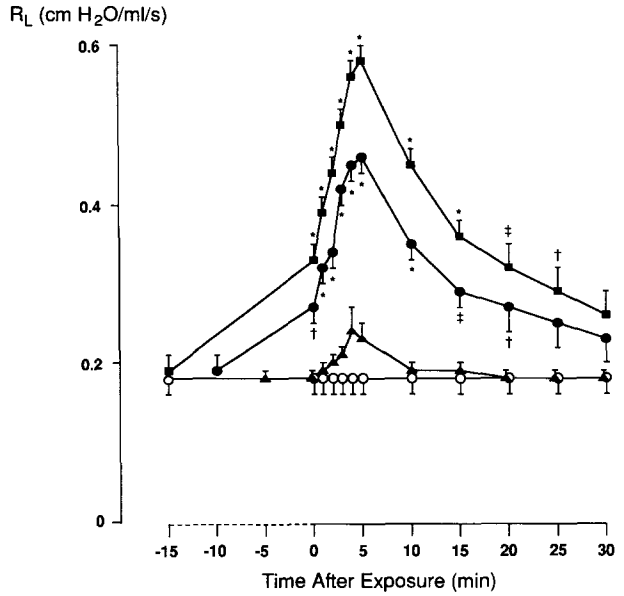

Fig. 1. Effect of inhalation of cold air on total pulmonary resistance (R_L) in anaesthetized guinea pigs. Baseline (open circles) indicates R_L induced by room air 15 min. Inhalation of cold air for 5 min (solid triangles) did not increase R_L above control, but longer periods of exposure increased R_L significantly. Increasing times of exposure to cold air (10 min (solid circles), 15 min (solid squares) caused an increase in R_L. Each point is the mean ± S.E.M. of five experiments. * $P < 0.001$, ‡ $P < 0.01$, † $P < 0.05$ vs. control (room air alone for 15 min)

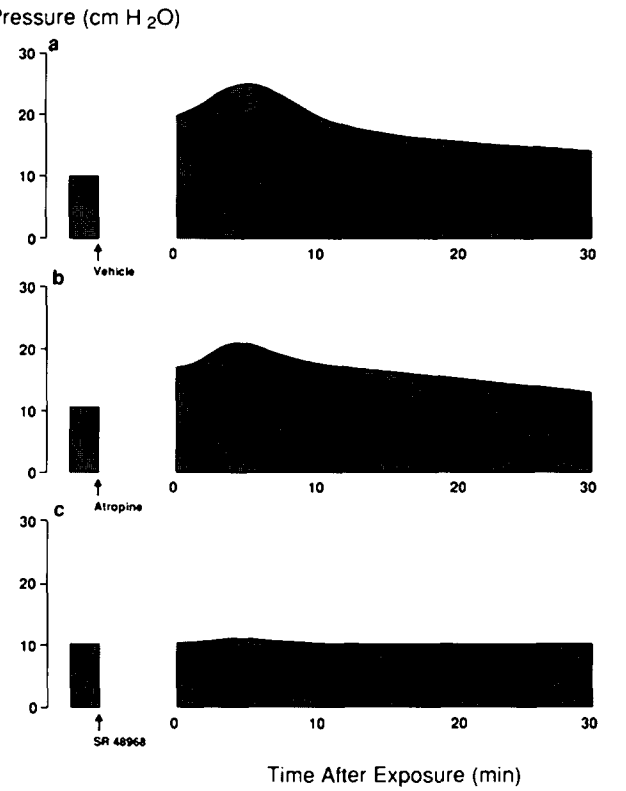

Fig. 2. Typical tracings showing the increase in intrapulmonary pressure (cm H_2O) produced by cold air inhalation for 10 min alone (a); after pretreatment with atropine (1.4 μmol/kg, i.v., 15 min before the exposure) (b); and after pretreatment with the tachykinin NK_2 receptor antagonist, SR 48968 (0.3 μmol/kg, i.v., 15 min before the exposure) (c) in anaesthetised guinea pigs.

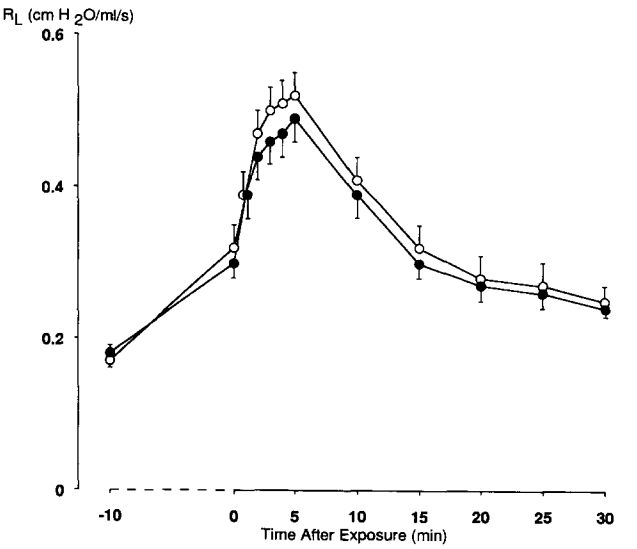

Fig. 3. Effect of atropine (solid circles; 1.4 μmol/kg, i.v., 15 min before the exposure) or vehicle (open circles; 0.9% saline) on the increase in total pulmonary resistance (R_L) induced by inhalation of cold air for 10 min in anaesthetised guinea pigs. Each point is the mean ± S.E.M. of seven experiments.

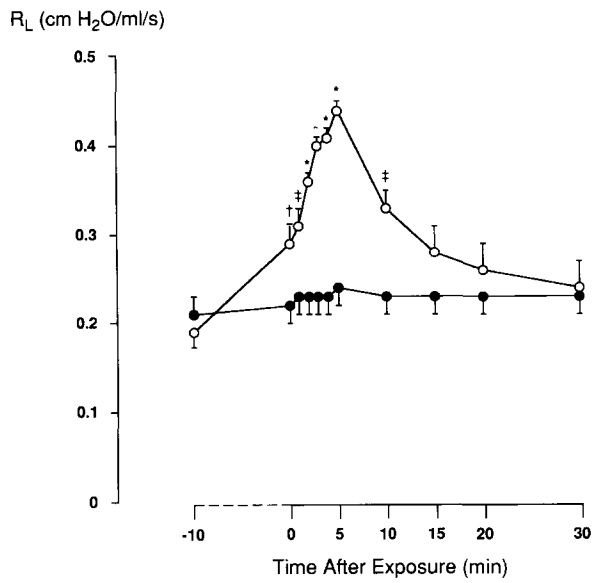

Fig. 4. Effect of the tachykinin NK_2 receptor antagonist (solid circles; SR 48968, 0.3 μmol/kg, i.v., 15 min before the exposure) on the increase in total pulmonary resistance (R_L) induced by inhalation of cold air for 10 min in anaesthetised guinea pigs. Each point is the mean ± S.E.M. of five experiments. * $P < 0.001$, ‡ $P < 0.01$, † $P < 0.05$ vs. control (open circles; cold air alone for 10 min).

The kinin B_2 receptor antagonist, HOE 140 (0.1 μmol/kg, i.v.) decreased the bronchoconstrictor response following inhalation of cold air (Fig. 6a). Pretreatment with a combination of histamine H_1 (pyrilamine, 10 mg/kg, i.p.) and histamine H_2 (cimetidine, 10 mg/kg, i.p.) receptor antagonists (Fig. 6b) or with the cyclooxygenase inhibitor, indomethacin (5 mg/kg, i.v.) had no significant effect on the bronchoconstrictor response to inhalation of cold air (Fig. 6c).

4. Discussion

The major finding of the present study is that exposure to cold air causes bronchoconstriction in guinea pigs, an effect that is due to the activation of sensory nerves and the release of tachykinins. In addition, the results indicate that kinin release plays a role in this response. Inhalation of cold air increased the maximum R_L and the duration of bronchoconstriction time dependently. This time-related response suggests that the degree of bronchoconstriction is somehow dependent upon the duration of the exposure of the airways to cold air. The increase in bronchoconstriction produced by cold air was not affected by atropine, thus indicating that a cholinergic efferent pathway does not play a role in this effect under the conditions of the present studies. However, the observation that the tachykinin NK_2 receptor antagonist, SR 48968 abolished the cold air-induced bronchoconstriction completely implicates tachykinins released from sensory nerve endings in the response. A further indication of the involvement of tachykinin release in the bronchoconstriction induced by the inhalation of cold air derives from the observation that inhibition by SR

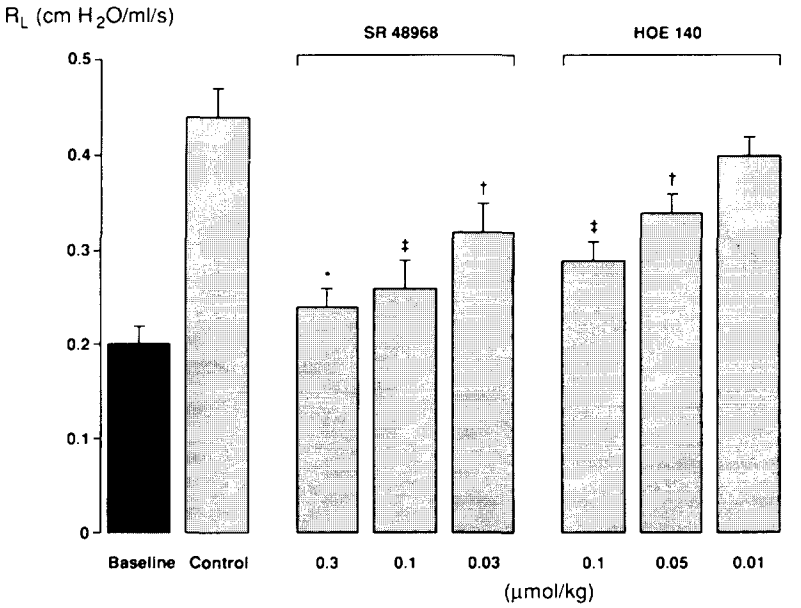

Fig. 5. Effect of pretreatment with the tachykinin NK_2 receptor antagonist (SR 48968, 0.03, 0.1 and 0.3 μmol/kg, i.v., 15 min before exposure) or the kinin B_2 receptor antagonist (HOE 140, 0.01, 0.05 and 0.1 μmol/kg, i.v., 15 min before exposure) on the increase in peak total pulmonary resistance (R_L) evoked by 10 min exposure to cold air in anaesthetised guinea pigs. Values are means ± S.E.M. of five experiments. * $P < 0.001$, ‡ $P < 0.01$, † $P < 0.05$ vs. control (cold air alone for 10 min).

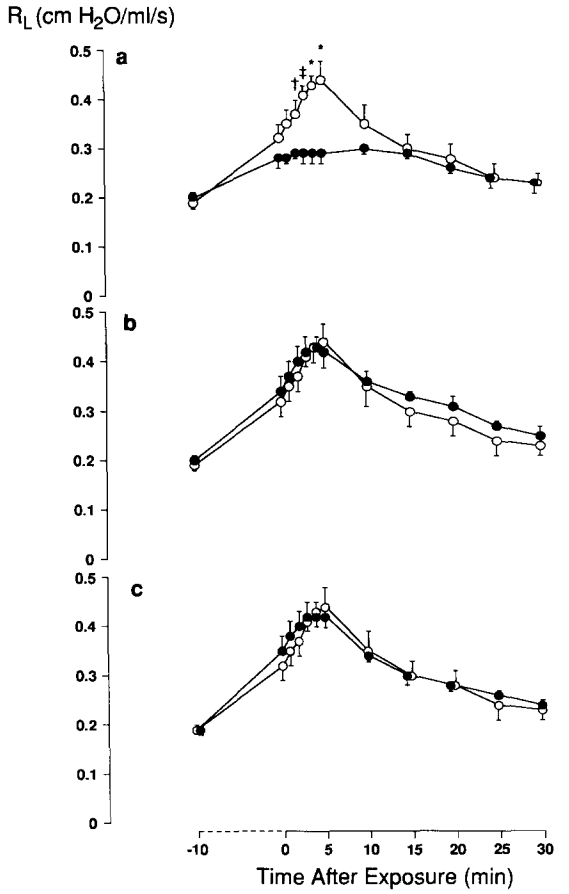

Fig. 6. Effect of the bradykinin B_2 receptor antagonist (HOE 140, 0.1 μmol/kg, i.v., 15 min before the exposure) (a; solid circles), a combination of H_1 (pyrilamine, 10 mg/kg, i.p., 30 min before exposure) and H_2 (cimetidine, 10 mg/kg, i.p., 30 min before exposure) histamine receptor antagonists (b; solid circles) or indomethacin (5 mg/kg, i.v., 30 min before exposure) (c; solid circles) on the increase in total pulmonary resistance (R_L) induced by inhalation of cold air for 10 min in anaesthetised guinea pigs. Each point is the mean ± S.E.M. of five experiments. $^{*}P < 0.001$, $^{\ddagger}P < 0.01$, $^{\dagger}P < 0.05$ vs. control (open circles; cold air alone for 10 min).

48968 and HOE 140 was dose-dependent. Tachykinin NK_2 receptor activation plays a major role in the bronchoconstriction evoked by endogenously released or exogenously administered tachykinins in guinea pigs (Maggi et al., 1991; Martins et al., 1991; Bertrand et al., 1993a). The ability of selective tachykinin NK_2 receptor antagonists to reduce or block bronchoconstriction evoked by antigen challenge (Bertrand et al., 1993b; Ricciardolo et al., 1994), hyperpnea (Solway et al., 1993), citric acid (Satoh et al., 1993), and metabisulfite (Sakamoto et al., 1994) has been used to establish that these stimuli increase bronchoconstriction in guinea pig airways by releasing tachykinins from sensory nerve endings.

Capsaicin selectively stimulates tachykinin-containing sensory nerves and can stimulate cold receptors belonging to both C- and Aδ-fibers (Seno and Dray, 1993). Previously, we reported that plasma extravasation induced by inhalation of cold air in the rat trachea is mediated by tachykinin release from sensory nerves (Yoshihara et al., 1995). From these observations we conclude that the bronchoconstriction induced by cold air is due to a similar mechanism described for cold air-induced plasma extravasation, i.e., the stimulation of sensory nerves and the subsequent release of tachykinins.

Prostanoids, kinins and histamine are among the mediators that are released during inflammation and that may exert their action in part by releasing sensory neuropeptides (Saria et al., 1983; Geppetti et al., 1991; Geppetti, 1993). The observation that indomethacin or a combination of histamine H_1 and H_2 receptor antagonists failed to reduce the bronchoconstriction induced by cold air is evidence against the involvement of histamine and prostanoids in the bronchoconstriction induced by cold air. The observation that HOE 140, a kinin B_2 receptor antagonist, reduced bronchoconstriction dose dependently following exposure to cold air, suggests that this exposure causes the release of kinins which subsequently cause at least part of the release of tachykinins from sensory nerve endings. HOE 140, at the maximal dose used in the present experiments (0.1 μmol/kg), has been shown previously to abolish bronchoconstriction induced by submaximal doses of bradykinin (Ricciardolo et al., 1994), suggesting that the bronchoconstriction induced by exposure to cold air is only partially due to kinin release.

Mediators other than prostanoids, histamine and kinins may be released by exposure of the airways to cold air, and these mediators may be responsible for the stimulation of sensory nerves and the subsequent release of tachykinins that results in bronchoconstriction. Alternatively, cold air may stimulate sensory nerves directly. Cold receptors are present in the airways. Nerve fibers conveying cold sensation (cold receptors) do not generally comprise polymodal nociceptors, and in particular the subpopulation of polymodal nociceptors sensitive to capsaicin and which contain neuropeptides. However, there are reports that some polymodal nociceptors may also be sensitive to cold stimuli (Seno and Dray, 1993).

During the cold season, asthma patients experience enhanced exercise-induced asthma (Strauss et al., 1977). Asthma-like symptoms, and bronchial hyperresponsiveness are much more common in cross-country skiers (who breathe large volumes of cold air) than in the general population and in non-skiers (Larsson et al., 1993). Weather changes may affect the airways by cooling and irritating them directly, or they may operate indirectly by influencing the level of airborne irritants and allergens. Exacerbation of asthma has been shown to correlate with low temperatures (Rossi et al., 1993). The observation that cold air exaggerates the

neurogenic inflammatory response in guinea pig airways suggests that, if a similar mechanism is present in humans, antagonists of tachykinin receptors may be of value in the reduction of bronchoconstriction during the cold season in preexisting inflammatory diseases of airways such as asthma.

Acknowledgements

This study was supported in part by National Institutes of Health Program Project Grant HL-06285.

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