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Acetylcholine and tachykinin receptor antagonists attenuate wood smoke-induced bronchoconstriction in guinea pigs

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Abstract

To study the mechanisms of wood smoke-induced bronchoconstriction, we measured total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) in anesthetized and mechanically ventilated guinea pigs. Airway exposure to various doses of wood smoke (lauan wood; 5, 10, and 15 breaths) resulted in a dose-dependent increase in R_L and decrease in C_{dyn}. The smoke-induced changes in R_L and C_{dyn} were significantly attenuated by pretreatment with atropine, CP-96,345 [(2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; a tachykinin NK_1 receptor antagonist], and SR-48,968 [(*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; a tachykinin NK_2 receptor antagonist] in combination, atropine alone, and SR-48,968 alone, but were not significantly affected by pretreatment with the inactive enantiomers of CP-96,345 and SR-48,968, CP-96,345 alone, indomethacin (a cyclooxygenase inhibitor), and MK-571 [((3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl((3-dimethyl amino-3-oxo-propyl)thio)methyl)propanoic acid; a leukotriene D_4 receptor antagonist]. The activity of airway neutral endopeptidase, a major enzyme for tachykinin degradation, was not significantly influenced by wood smoke during the development of bronchoconstriction. We conclude that: (1) both cholinergic mechanisms and endogenous tachykinins, but not cyclooxygenase products or leukotriene D_4, play an important role in the acute bronchoconstriction induced by wood smoke, and (2) the contribution of tachykinins to this airway response is primarily mediated via the activation of tachykinin NK_2 receptors, but is not associated with inactivation of the airway neutral endopeptidase.

Keywords: Respiratory irritant; Bronchoconstriction; Tachykinin; Cholinergic reflex; Neutral endopeptidase

1. Introduction

Acute airway exposure to toxic smoke generated by the incomplete combustion of materials such as wood or polymer is known to cause bronchoconstriction in humans (Petroff et al., 1976; Haponik and Munster, 1986) and laboratory animals (Traber and Herndon, 1986; Prien et al., 1988; Wang et al., 1990; Loick et al., 1993). The smoke-induced airway constriction may possibly produce a severe bronchospasmodic attack in individuals at the fire site and is likely to contribute to various pathophysiological consequences observed in fire victims, including airway obstruction, ventilation–perfusion mismatching, and respiratory insufficiency (Crapo, 1981; Haponik and Munster, 1986; Traber and Herndon, 1986). Although several investigators have postulated that centrally mediated cholinergic reflexes (Prashad et al., 1979; Crapo, 1981; Cohen and Guzzardi, 1983) and chemical mediators released locally in the lungs (Traber and Herndon, 1986; Prien et al., 1988; Loick et al., 1993) may be involved in evoking bronchoconstriction following toxic smoke inhalation, the pathophysiological mechanisms are still largely unclear.

We recently reported that inhaled wood smoke stimulates irritant receptors (Lai and Kou, 1998a) and vagal C-fiber nerve endings located in the lungs (Lai and Kou, 1998b). Stimulation of the former type of pulmonary receptors can trigger reflex bronchoconstriction (Karlsson et al., 1988), whereas activation of the latter type is known to evoke bronchoconstriction mediated through cholinergic reflex mechanisms and/or the release of tachykinins

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(Karlsson et al., 1988; Solway and Leff, 1991; Barnes, 1996). Tachykinins are sensory neuropeptides released locally from airway afferent C-fiber nerve endings in response to chemical irritants (Solway and Leff, 1991; Advenier, 1995; Barnes, 1996). Tachykinins such as substance P and neurokinin A are known to cause potent bronchoconstrictive effects by activation of tachykinin NK_1 and NK_2 receptors, respectively (Solway and Leff, 1991; Advenier, 1995; Barnes, 1996). The released tachykinins are rapidly degraded by the neutral endopeptidase, a cell membrane-bound peptidase that is present in the airways and lungs (Solway and Leff, 1991; Barnes, 1996). Inhibition of this enzyme may potentiate the bronchoconstrictive effects of tachykinins (Solway and Leff, 1991; Barnes, 1996). It is known that wood smoke contains many chemical toxins, such as ozone, acrolein, and free radicals (Pryor, 1992; Larson and Koenig, 1994), which have been shown to inhibit airway neutral endopeptidase (Solway and Leff, 1991; Turner et al., 1993; Barnes, 1996). Taken together, these findings suggest the possibility that cholinergic mechanisms, tachykinins, and inactivation of airway neutral endopeptidase may be involved in evoking smoke-induced bronchoconstriction. However, experimental support for this possibility remains to be established.

In addition to tachykinins, inhalation of toxic smoke has been shown to cause an increase in the release of other chemical mediators such as cyclooxygenase (i.e., prostaglandins and thromboxane) and lipoxygenase products (i.e., leukotrienes) (Traber and Herndon, 1986; Quinn et al., 1990; Hales et al., 1995). These chemical mediators are arachidonate metabolites, many of which possess potent bronchoconstrictive effects (Bakhle and Ferreira, 1986). However, the role of arachidonate metabolites in producing smoke-induced airway constriction has not been clearly defined.

This study on anesthetized guinea pigs was undertaken to determine (1) the dose–response relationship of acute bronchoconstrictive responses to inhaled wood smoke, (2) the relative contribution of cholinergic mechanisms, tachykinins, and arachidonate metabolites to the smoke-induced airway constriction, and (3) whether this airway constriction is associated with a smoke-induced reduction in the activity of airway neutral endopeptidase.

2. Materials and methods

2.1. Animal preparation

Male Hartley guinea pigs weighing 300–365 g (averaging 327 ± 2 g; $n = 90$) were anesthetized with an intraperitoneal injection of chloralose (100 mg kg^{-1}; Sigma Chemical, St. Louis, MO, USA) and urethane (500 mg kg^{-1}; Sigma). The carotid artery and jugular vein were cannulated for recording arterial blood pressure and for intravenous administration of pharmacological agents, respectively. During the course of the experiments, supplemental doses of chloralose (20 mg kg^{-1} h^{-1}) and urethane (100 mg kg^{-1} h^{-1}) were administered to maintain abolition of pain reflexes induced by pinching the animal's hindpaw. The animals were tethered in a supine position and the trachea was cannulated below the larynx with a short tracheal cannula via a tracheotomy, through which the animals were ventilated with a rodent respirator (model 683, Harvard Apparatus, South Natick, MA, USA) at the constant rate of 60 breaths min^{-1}. Tidal volume (V_T) was adjusted according to the body weight of each animal (10 ml kg^{-1}) and was kept constant in each experiment. The animals were then paralyzed with an intravenous injection of pancuronium bromide (0.05 mg kg^{-1}; Organon Teknika, Boxtel, Holland). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked. Throughout the experiment, the body temperature of the animals was maintained at ~ 36°C by means of a servo heating blanket. All protocols were in accordance with the guidelines for the care and use of laboratory animals published by the National Institutes of Health (Bethesda, MD, USA) and were approved by the Committee of National Science Council (Taipei, Taiwan).

2.2. Measurements of bronchomotor responses

Intrapleural pressure was measured by a catheter inserted into the right intrapleural cavity via a surgical incision between the fifth and sixth ribs. The incision was subsequently sutured and further sealed with silicone jelly. The pneumothorax caused by this procedure was corrected by briefly opening the intrapleural catheter to ambient air during a held hyperinflation ($4 \times V_T$). Tracheal pressure was measured by another catheter connected to the side arm of the tracheal cannula. Transpulmonary pressure (P_{tp}) was measured as the difference between tracheal pressure and intrapleural pressure, using a differential pressure transducer (P300D, Validyne, Northridge, CA, USA). Respiratory flow was measured with a pneumotachograph (No. 4/0, Fleisch, Richmond, VA, USA) coupled to a differential pressure transducer (MP45-14, Validyne) and was integrated to give V_T. The pneumotachograph was positioned in-line with the respirator. All physiological signals were recorded simultaneously with a chart recorder (RS3200, Gould, Cleveland, OH, USA) and a tape recorder (DR-890, Neurocorder, New York, NY, USA), and were analyzed with an on-line computer system. Total lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were measured on a breath-by-breath basis with a computer equipped with an analog/digital convertor (DASA 4600, Gould) and a software (version 1.1, BioCybernatics, Taipei, Taiwan), using the subtraction method. Results obtained from the computer analysis were routinely checked for accuracy with those calculated manually.

2.3. Generation and delivery of wood smoke

The electric furnace and the methods for generating wood smoke are described in detail in our previous study (Kou and Lai, 1994). In brief, 100 g of dry wood dust (lauan wood) was thermally decomposed by the furnace (model 101, Nan Jou, Taipei, Taiwan) at a core temperature maintained at 500 ± 8°C for 5 min and the effluent smoke was collected in a 25-l plastic balloon attached to the furnace outlet. Wood smoke was sampled and analyzed for its O_2 (OM-11, Beckman, Fullerton, CA, USA), CO_2 (LB-2, Beckman), CO (model 961, Neotronics, UK) and particulate (model P-5H2, Sibata, Tokyo, Japan) concentrations. Wood smoke generated by this method contains approximately 1.5% O_2, 15% CO_2, 24% CO, and 25 mg l^{-1} particulates (Kou et al., 1995). Immediately after smoke generation, the plastic balloon containing the smoke was then attached to the inspiratory inlet of the respirator via a 3-way stopcock. Wood smoke at a temperature of ~25°C was delivered into the lungs when the 3-way stopcock was turned to communicate the respirator with the balloon. Before each smoke challenge, the lungs were hyperinflated ($4 \times V_T$) to establish a constant volume history. To avoid contamination, the expired smoke was drawn into the fume hood via a suction line.

2.4. Pharmacological pretreatments

An acetylcholine receptor antagonist (atropine sulfate, 1 mg kg^{-1}; Sigma) and a leukotriene D_4 receptor antagonist [MK-571, 2 mg kg^{-1}; ((3-(3-(2-(7-chloro-2-quinolinyl) ethenyl)phenyl((3-dimethyl amino-3-oxo-propyl)thio)-methyl)propanoic acid; Cayman Chemical, Ann Arbor, MI, USA] were dissolved in isotonic saline to concentrations of 1 mg ml^{-1} and 2 mg ml^{-1}, respectively. A tachykinin NK_1 receptor antagonist [CP-96,345, 2 mg kg^{-1}; (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; Pfizer, Groton, CT, USA], an inactive enantiomer of CP-96,345 [CP-96,344, 2 mg kg^{-1}; (2*R*,3*R*)-*cis*-2-(diphenylmethyl)-*N*-((2-methoxyphenyl)-methyl)-1-azabicyclo(2.2.2.)-octan-3-amine; Pfizer], a tachykinin NK_2 receptor antagonist [SR-48,968, 2 mg kg^{-1}; (*S*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; Sanofi Research, Montpellier, France], and an enantiomer of SR-48,968 [SR-48,965, 2 mg kg^{-1}; (*R*)-*N*-methyl-*N*(4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl)benzamide; Sanofi] were separately dissolved in 50% ethanol and then diluted in isotonic saline to a final concentration of 2 mg ml^{-1}. A cyclooxygenase inhibitor (indomethacin, 5 mg kg^{-1}; Sigma) was first

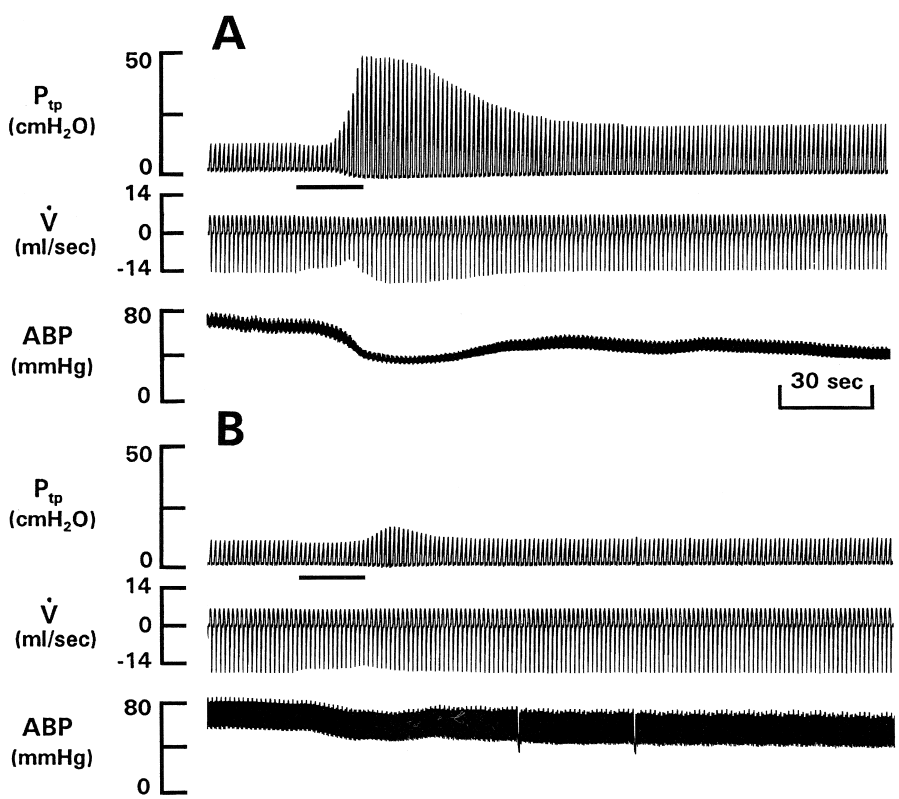

Fig. 1. Experimental tracings illustrating responses of transpulmonary pressure (P_{tp}), respiratory flow ($\dot{V}$), and arterial blood pressure (ABP) to 15 tidal breaths of wood smoke in two anesthetized and mechanically ventilated guinea pigs. (A) A control animal; (B) An animal pretreated with atropine, CP-96,345, and SR-48,968 in combination. Wood smoke was delivered into the lungs by a respirator as indicated by the horizontal bars.

dissolved in polyethylene glycol and then diluted in a 1:1 ratio with saline to a final concentration of 5 mg ml^{-1}. These drugs were injected as a bolus into the vein. The doses of these drugs are equal to or greater than those previously used by other investigators (Sakamoto et al., 1993; Hong et al., 1995; Hey et al., 1996; Yang et al., 1997) in the study of bronchoconstriction induced by other experimental conditions in guinea pigs.

2.5. Experimental procedures

A total of 90 animals were randomly and equally divided into 15 groups. In Study 1, bronchoconstrictive responses to the challenge air and to challenge with 5, 10, or 15 tidal breaths of wood smoke were investigated in four groups of animals. In Study 2, bronchoconstrictive responses to 15 tidal breaths of wood smoke with the following pharmacological pretreatments were investigated in nine groups of animals. Each of these nine groups received a pretreatment with atropine, CP-96,345, and SR-48,968 in combination, CP-96,344 and SR-48,965 in combination, atropine alone, CP-96,345 alone, SR-48,968 alone, indomethacin alone, MK-571 alone, vehicle of CP-96,345 and SR-48,968, or vehicle of indomethacin 15 min before the smoke challenge. In both Studies 1 and 2, R_L was monitored 1 min before and at least 2.5 min after each smoke challenge. Immediately after this observation period, the lungs were hyperinflated ($4 \times V_T$) two times to restore R_L and C_{dyn} to their normal baseline values. In Study 3, one group of animals received a sham air challenge, while the other group received 15 tidal breaths of wood smoke. Thirty seconds after the air or smoke challenge, the airway tissues including the tracheal segment below the tip of the tracheal cannula and the main stem bronchus were quickly removed, washed with isotonic saline, and then stored at −70°C for analysis of neutral endopeptidase activity.

2.6. Determination of airway neutral endopeptidase activity

The activity of airway neutral endopeptidase was determined as described previously (Lai and Chiang, 1997; Hsu et al., 1998). Briefly, the frozen airway tissues were thawed and minced in tubes containing 50 mM Tris, pH 7.4. The tissues were then sonicated for 30 s at 4°C and centrifuged at $17,500 \times g$ for 15 min. The supernatant was removed

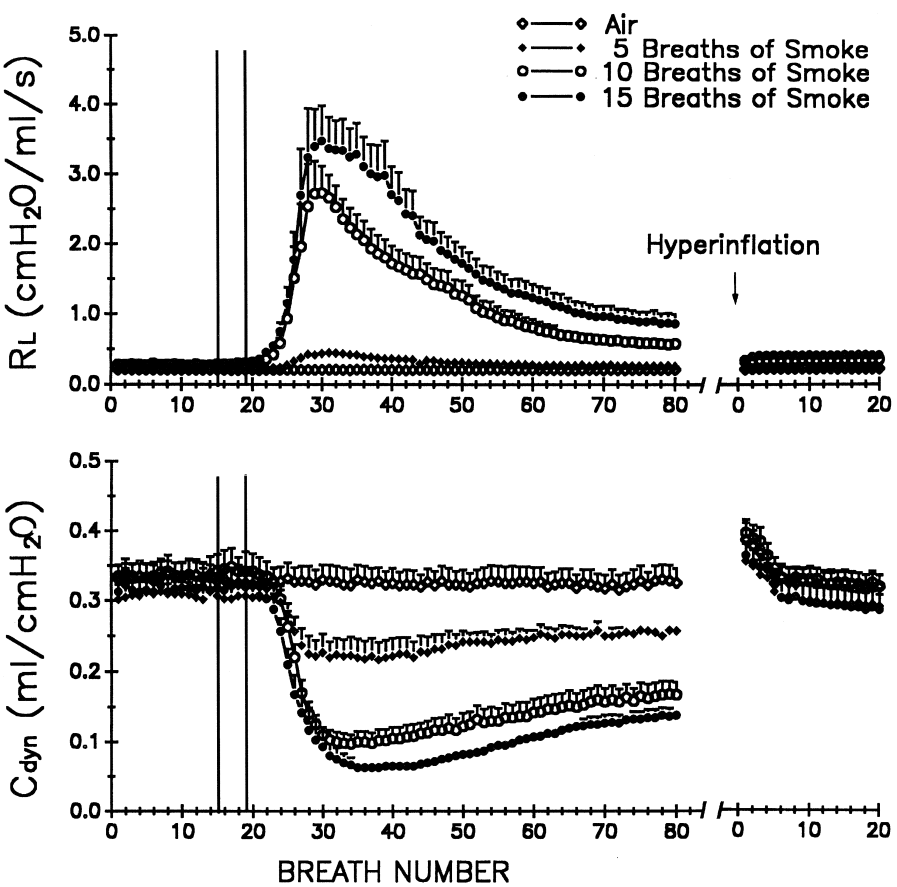

Fig. 2. Bronchomotor responses to air and three doses of wood smoke in four groups of animals. Wood smoke was delivered into the lungs by a respirator. The two vertical lines indicate the period of smoke delivery. Hyperinflation of the lungs was produced by occluding the expiratory line of the respirator for four consecutive cycles and was performed immediately after the 2.5-min observation period. R_L, total lung resistance; C_{dyn}, dynamic lung compliance. Data in each group are means ± S.E. for six animals. Note that R_L and C_{dyn} were restored to their normal baseline values following hyperinflation.

for analysis. In the presence of substrate, the amount of 2-naphthylaminie released by the tissues was determined, using a spectrophotometer (model Lambda 3B, Perkin-Elmer, USA) at 530 nm. The phosphoramidon-inhibitable neutral endopeptidase specific activity was expressed as nanomoles per milligram of tissue protein per hour.

2.7. Statistical analysis

The baseline R_L was calculated as the value averaged over the 10-breath period immediately preceding the smoke challenge. Peak responses were measured as the peak increase in R_L or maximal reduction in C_{dyn} averaged over 5 consecutive breaths after the smoke challenge. Peak responses are expressed as percent changes from baseline values in R_L or C_{dyn}. Data for changes in R_L or C_{dyn} were analyzed by a one-factor analysis of variance followed by Fisher's least significant difference procedure when appropriate. Data for airway neutral endopeptidase activity were analyzed by a Student's *t*-test. $P < 0.05$ was considered significant. All data are presented as means ± S.E.

3. Results

3.1. Baseline data of the animals studied

In a total of 90 animals studied, the baseline R_L and C_{dyn} were 0.27 ± 0.01 cm H_2O/ml/s and 0.31 ± 0.01 ml/H_2O, respectively. There were no statistical differences ($P > 0.05$) in body weight, baseline R_L, and baseline C_{dyn} among the groups of animals studied.

3.2. Smoke-induced bronchoconstriction: dose–response relationship

In control animals, delivery of 15 breaths of wood smoke into the lungs induced a rise in P_{tp}, which started to increase within 6–9 breaths, reached its peak in 12–18 breaths after smoke challenge, and then gradually declined, but remained at a level higher than its baseline value for > 2.5 min (e.g., Fig. 1A). In a similar time course, R_L increased and C_{dyn} decreased; both reached maximal changes within 11–20 breaths after smoke delivery and then gradually returned toward their baseline values (Fig. 2). Manual hyperinflation of the lungs ($4 \times V_T$) totally reversed the residual elevation of P_{tp} (e.g., Fig. 1A) and reduction in C_{dyn} (Fig. 2) which remained at the end of the 2.5-min observation period. Compared to 15 breaths of wood smoke, delivery of 5 or 10 breaths of wood smoke produced bronchoconstrictive responses of smaller magnitude and shorter duration (Fig. 2). In contrast to these smoke effects, delivery of air did not cause any detectable change in R_L or C_{dyn} (Fig. 2). Averaged peak changes in R_L and C_{dyn} are shown in Fig. 3. Statistical analysis

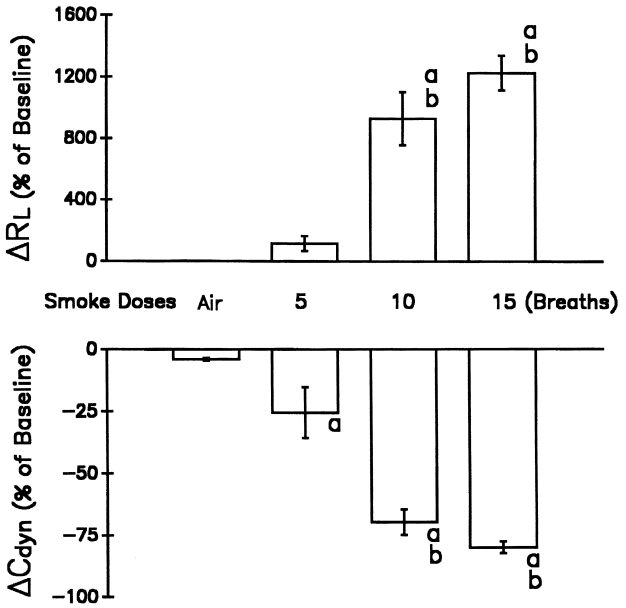

Fig. 3. Dose–response relationship of peak increase in total lung resistance (R_L) and maximal reduction in dynamic lung compliance (C_{dyn}) induced by various doses of wood smoke in four groups of animals. [a]Significantly ($P < 0.05$) different from the response to air challenge; [b]Significantly ($P < 0.05$) different from the response to 5 tidal breaths of wood smoke. Data in each group are means ± S.E. for six animals.

revealed that both 10 and 15 breaths of wood smoke, but not 5 breaths of wood smoke, induced a significant increase in R_L (Fig. 3). Furthermore, all three doses of wood smoke caused a significant decrease in C_{dyn}. The decrease induced by 10 or 15 breaths of wood smoke was significantly greater than that induced by 5 breaths of wood smoke (Fig. 3). However, the changes in R_L and C_{dyn} induced by 15 breaths of wood smoke were not significantly different from those induced by 10 breaths of wood smoke (Fig. 3). The responses to 15 breaths of wood smoke were then taken as the standard responses for subsequent studies in animals pretreated with various pharmacological agents.

3.3. Effects of pharmacological pretreatments on smoke-induced bronchoconstriction

In animals pretreated with either atropine, CP-96,345, and SR-48,968 in combination (Fig. 1B), atropine alone, or SR-48,968 alone, the bronchoconstrictive responses to 15 breaths of wood smoke were significantly alleviated. In contrast, animals pretreated with either CP-96,344 and SR-48,965 in combination, CP-96,345 alone, indomethacin alone or MK-571 alone still displayed full smoke-induced bronchoconstriction. On average, the smoke-induced changes in R_L and C_{dyn} were significantly attenuated by atropine, CP-96,345, and SR-48,968 in combination, atropine alone, or SR-48,968 alone, but not by CP-96,344 and SR-48,965 in combination, CP-96,345 alone, indomethacin alone, MK-571 alone, vehicle of CP-96,345 and

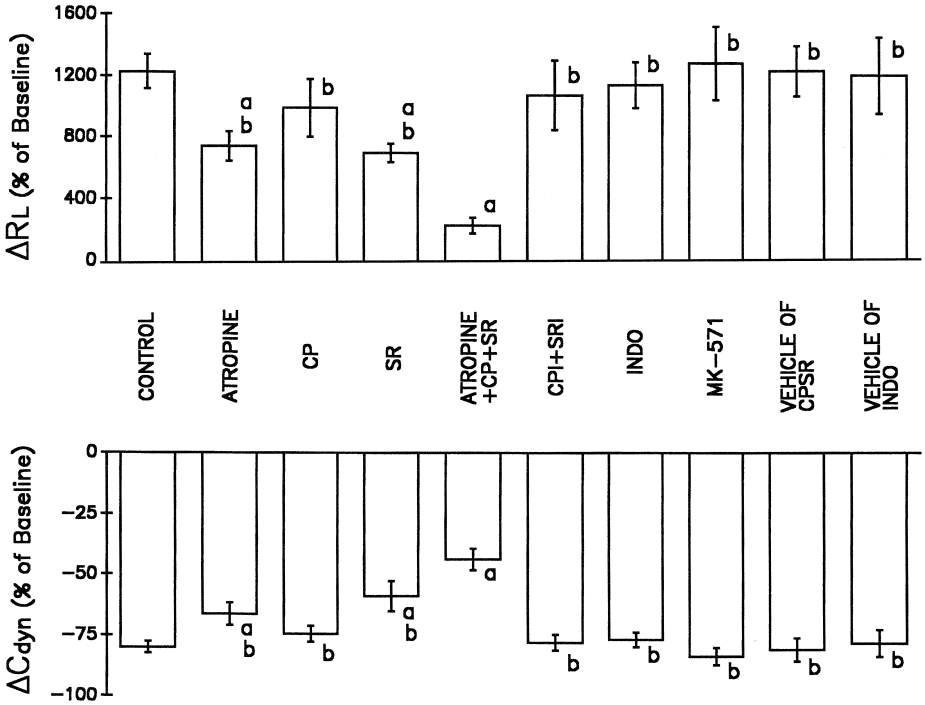

Fig. 4. Peak increase in total lung resistance (R_L) and maximal reduction in dynamic lung compliance (C_{dyn}) in response to 15 tidal breaths of wood smoke in control animals and in nine groups of animals pretreated with atropine alone, CP-96,345 alone (CP), SR- 48,968 alone (SR), atropine, CP-96,345, and SR-48,968 in combination (ATROPINE + CP + SR), CP-96,344 and SR-48,965 in combination (CPI + SRI), indomethacin alone (INDO), MK-571 alone, vehicle of CP-96,345 and SR-48,968 (VEHICLE OF CPSR), or vehicle of indomethacin (VEHICLE OF INDO). Pretreatments were made 15 min before smoke challenge. [a]Significantly ($P < 0.05$) different from responses measured in control animals; [b]Significantly ($P < 0.05$) different from responses measured in animals pretreated with ATROPINE + CP + SR. Data in each group are means ± S.E. for six animals.

SR-48,968, or vehicle of indomethacin (Fig. 4). Additionally, atropine, CP-96,345, and SR-48,968 in combination caused a significantly larger suppression of the bronchoconstriction in comparison to that produced by either atropine alone, CP-96,345 alone, or SR-48,968 alone (Fig. 4).

3.4. Effects of wood smoke on airway neutral endopeptidase activity

The activity of airway neutral endopeptidase measured in tissues excised 30 s after smoke delivery (44.7 ± 5.7 nmol mg protein^{-1} h^{-1}) was not significantly different from that in tissues excised at the same time after sham air challenge (41.2 ± 5.0 nmol mg protein^{-1} h^{-1}; $P > 0.05$, $n = 6$).

4. Discussion

In anesthetized, mechanically ventilated, closed chest guinea pigs, we demonstrated that the delivery of various doses of wood smoke into the airways evoked acute bronchoconstriction in a dose-dependent manner. The acute smoke-induced bronchoconstriction was evidenced by a prominent increase in R_L with a concomitant decrease in C_{dyn}. The deleterious effects of inhaled toxic smoke on lung mechanics have been repeatedly demonstrated in both clinical and animal studies, in which the time lag before assessment of pulmonary functions varied from several minutes to a few days after smoke inhalation (Petroff et al., 1976; Prashad et al., 1979; Nieman et al., 1980; Prien et al., 1988; Wang et al., 1990; Loick et al., 1993). The present study demonstrates that dramatic changes in lung mechanics occur as rapidly as a few seconds after smoke inhalation. It has been suggested that an increase in R_L is indicative primarily of constriction in central airways because these airways normally account for 90% or more of the total resistance to airflow in the tracheobronchial tree (Macklem and Mead, 1976). Conversely, C_{dyn} is an index of tone in peripheral airways and lung parenchyma, and its decrease can be brought about by several mechanisms including nonuniform constriction of airways (Drazen and Austen, 1974). Our finding that the smoke-induced reduction in C_{dyn} could be totally reversed by hyperinflation of the lungs (Fig. 2) suggests a possible involvement of lung atelectasis resulting from partial closure of small airways. Indeed, Nieman et al. (1980) observed the development of gross atelectasis of the lungs in as few as 2 breaths (8 s) after wood smoke inhalation in an open-chest dog model, despite the presence of post end-expiratory pressure, and suggested that primary airway constriction might be operative. Accordingly, our results might indicate that inhaled wood smoke exerts its bronchoconstricitve effects on both central airways and the peripheral area of the lungs.

We further demonstrated that pretreatment with a combination of atropine, CP-96,345, and SR-48,968 greatly reduced the bronchoconstrictive responses to inhaled wood smoke, whereas pretreatment with a combination of inactive enantiomers of CP-96,345 and SR-48,968 failed to do so. CP-96,345 and SR-48,968 are selective tachykinin NK_1 and NK_2 receptor antagonists, respectively (Snider et al., 1991; Edmonds-Alt et al., 1992; Advenier, 1995). To delineate the relative contribution of cholinergic mechanisms and tachykinins, we selectively blocked muscarinic, tachykinin NK_1, and tachykinin NK_2 receptors with their specific antagonists. We found that pretreatment with either atropine or SR-48,968 significantly alleviated the smoke-induced bronchoconstriction, while pretreatment with CP-96,345 did not produce any significant effect. These results suggest that both cholinergic mechanisms and endogenous tachykinins play an important role in producing acute smoke-induced bronchoconstriction, and that the contribution of tachykinins is primarily mediated through the activation of tachykinin NK_2 receptors. The cholinergic bronchoconstriction may possibly be elicited by the stimulation of lung irritant receptors and bronchopulmonary C-fiber nerve endings by inhaled wood smoke (Lai and Kou, 1998a,b) and/or by the activation of arterial chemoreceptors by the extremely low oxygen and high carbon dioxide concentrations in the smoke (Kou and Lai, 1994). The contribution of tachykinins to the observed airway responses may originate from an increase in their release from bronchopulmonary C-fiber nerve endings when wood smoke stimulates these pulmonary receptors (Lai and Kou, 1998b) and/or may result from the smoke-induced inactivation of airway neutral endopeptidase, the major enzyme for tachykinin degradation (Solway and Leff, 1991; Barnes, 1996). However, we found no evidence of the involvement of neutral endopeptidase inactivation in the acute smoke-induced bronchoconstriction observed in this study because neutral endopeptidase activity was not significantly affected in airway tissues excised 30 s after smoke delivery, a time when these immediate changes in lung mechanics occurred. Among the various effects of tachykinins in guinea pig airways, plasma extravasation is predominantly mediated through the activation of tachykinin NK_1 receptors, whereas airway smooth muscle contraction is mainly due to the activation of tachykinin NK_2 receptors (Advenier, 1995; Barnes, 1996). Hence, our finding that tachykinin NK_2 receptors are important in producing the smoke-induced airway constriction is not surprising. Indeed, the significance of tachykinins in the bronchoconstrictive responses to other respiratory irritants such as cigarette smoke (Wu et al., 1997) or SO_2 (Hajj et al., 1996) has also been shown to be mediated mainly through the activation of tachykinin NK_2 receptors.

Several studies have suggested the possibility that endogenous tachykinins may act on their receptors located in the parasympathetic ganglia to facilitate cholinergic bronchoconstriction (Watson et al., 1993; Hey et al., 1996). Therefore, it is possible that the functional importance of cholinergic mechanisms and tachykinins in producing smoke-induced airway constriction is interrelated. However, the fact that pretreatment with atropine, CP-96,345, and SR-48,968 in combination provided a more complete blockade of the smoke-induced bronchoconstriction then did pretreatment with either atropine alone or SR-48,968 alone (Fig. 4) suggests that these two contributory factors may act, at least in part, independently to produce the observed airway response. Furthermore, the effects of atropine alone, SR-48,968 alone, or atropine, CP-96,345, SR-48,968 in combination appeared to be more pronounced on the increase in R_L than on the decrease in C_{dyn} (Fig. 4), implying that both cholinergic mechanisms and tachykinins may contribute more to the central airway response than to the peripheral airway response.

Pretreatment with a combination of atropine, CP-96,345, and SR-48,968 reduced the smoke-induced increase in R_L by 80% and reversed the decrease in C_{dyn} by 45%. The incomplete blockade of bronchoconstriction could be due to insufficient doses of these drugs. Since wood smoke contains numerous irritant gases (Larson and Koenig, 1994), the possibility that the residual airway responses observed in animals pretreated with acetylcholine and tachykinin receptor antagonists were due to the direct chemical irritation of airway smooth muscles (Crapo, 1981) should be considered. Alternatively, it is plausible that other types of chemical mediators are involved. Because inhaled toxic smoke has been shown to cause an increase in the release of cyclooxygenase and lipoxygenase products (Traber and Herndon, 1986; Quinn et al., 1990; Hales et al., 1995), we then focused on the possible involvement of these arachidonate metabolites. However, pretreatment with either indomethacin (a cyclooxygenase inhibitor) or MK-571 (a leukotriene D_4 receptors antagonist) failed to modify the smoke-induced bronchoconstriction, suggesting that cyclooxygenase products and leukotriene D_4 are unlikely to play a vital role. In a sheep model, Loick et al. (1993) observed a delayed-onset increase in airway peak pressure 12 h after smoke inhalation, which could be prevented by pretreatment with a thromboxane receptor antagonist. Thus, different chemical mediators may be involved in producing bronchoconstriction developed at different stages of inhalation pathophysiology. Clearly, more studies are required to better understand the exact mechanisms underlying the airway constriction induced by toxic smoke.

The other method to accomplish the objectives of this study is to compare the bronchoconstrictive responses to inhaled wood smoke before and after pharmacological pretreatment in the same animals, providing that two repeated smoke challenges could evoke reproducible responses in the control animals. However, these within-group comparisons could not be made because, in a parallel study (Hsu et al., 1998), we found that prior airway

exposure of 4 tidal breaths of wood smoke induced an increase in airway responsiveness to subsequent smoke inhalation in guinea pigs. Hence, although the same amount of smoke was delivered to the lungs, the bronchoconstrictive responses evoked by the second smoke challenge were 4.3-fold greater than those evoked by the first challenge in the same animals. The magnitude of the bronchoconstriction evoked by delivery of 15 tidal breaths of wood smoke in this study was comparable to that evoked by the second challenge of only 4 tidal breaths of wood smoke in our previous study. The model of a single airway exposure to a large dose of toxic smoke in this study may mimic the pattern of smoke inhalation in fire victims, whereas the model of recurrent airway exposure to a small dose of toxic smoke in the previous study may simulate the pattern of smoke inhalation in fire fighters. Accordingly, results obtained from this and the previous study (Hsu et al., 1998) are relevant to the hypothesis that both the pattern and the amount of toxic smoke inhalation are important determinants of the magnitude of evoked bronchoconstriction.

In conclusion, these results show that both cholinergic mechanisms and endogenous tachykinins, but not arachidonate metabolites, play an important role in producing acute smoke-induced bronchoconstriction in guinea pigs. Additionally, we show that the contribution of tachykinins to these bronchoconstrictive responses is primarily mediated via the activation of tachykinin NK_2 receptors and is not associated with inactivation of the airway neutral endopeptidase.

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