Cigarette smoke-induced bronchoconstriction: causative agents and role of thromboxane receptors

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Hong, Ju-Lun, and Lu-Yuan Lee. Cigarette smoke-induced bronchoconstriction: causative agents and role of thromboxane receptors. J. Appl. Physiol. 81(5): 2053–2059, 1996.—Inhalation of cigarette smoke induces a biphasic bronchoconstriction in guinea pigs: the first phase is induced by a combination of cholinergic reflex and tachykinins, whereas the second phase involves cyclooxygenase metabolites. Inhalation of 10 ml of high-nicotine cigarette smoke consistently elicited the biphasic bronchoconstriction in anesthetized and artificially ventilated guinea pigs. Pretreatment with hexamethonium (10 mg/kg iv) significantly reduced the first-phase bronchoconstriction but did not have any measurable effect on the second-phase response. In sharp contrast, gas-phase smoke did not elicit any bronchoconstrictive effect. Furthermore, when the animals were challenged with low-nicotine cigarette smoke, only a single second-phase response was evoked, accompanied by increases in thromboxane (TX)A2 (a stable metabolite of TxA2), prostaglandin (PG)D2, PGF2α, and thromboxane (TX)B2 (a stable metabolite of TxA2), prostaglandin (PG)D2, PGF2α, in the bronchoalveolar lavage fluid. The bronchoconstrictive response induced by low-nicotine smoke was completely prevented by pretreatment with SQ-29548 (0.3 mg/kg iv), a TxA2-receptor antagonist. These results indicate that 1) nicotine is the primary causative agent responsible for the first-phase bronchoconstriction and 2) nonnicotine smoke particulates evoke the release of TxA2, PGD2, and PGF2α, which act on TxA2 receptors on airway smooth muscles and induce the second-phase response to cigarette smoke.

hexamethonium; SQ-29548; tachykinins; guinea pigs; prostanoids

INHALATION OF CIGARETTE SMOKE has repeatedly been shown to induce acute bronchoconstriction in various species including humans (9, 15, 20). In a previous study, Hong et al. (9) have reported that inhalation of cigarette smoke in guinea pigs induces an acute bronchoconstriction consisting of two distinct phases that are different in both the time course and the underlying mechanism; the first phase is induced by a combination of cholinergic reflex and tachykinins release, whereas the second phase is inhibited by a pretreatment with indomethacin, suggesting the involvement of arachidonic acid metabolites of the cyclooxygenase pathway. Several questions regarding the smoke-induced bronchoconstriction remained unanswered in that study. First, although nicotine has been shown to be the major causative agent in cigarette smoke responsible for activating bronchopulmonary sensory afferents and for inducing an immediate bronchoconstriction via the cholinergic reflex in dogs (8, 11, 14), the smoke constituent(s) responsible for the release of tachykinins and cyclooxygenase metabolites in guinea pigs has not yet been identified. Second, two types of prostanoid receptors, thromboxane (TX)A2-sensitive (TP) receptors and one subtype of prostaglandin (PG) E2-sensitive (EP3) receptors (3, 18) that are known to mediate bronchoconstriction, have been identified in the guinea pig airways; the receptor types and the bronchoconstrictive prostanoids responsible for the smoke-induced bronchoconstriction have not been elucidated.

Therefore, the purposes of this study were 1) to identify the cigarette smoke constituents that induce the first- and second-phase responses and 2) to determine the types of prostanoid receptors and endogenous prostanoids involved in the smoke-induced second-phase bronchoconstriction.

METHODS

Male Hartley guinea pigs, ranging from 330 to 580 g body weight, were anesthetized with an intraperitoneal injection of a-chloralose (100 mg/kg) and urethan (500 mg/kg). The trachea was cannulated below the larynx via a tracheostomy with a tracheal cannula through which the animal was ventilated with a respirator (Harvard model 683) at a constant rate of 44 breaths/min. Tidal volume (VT) was adjusted according to the body weight (8 ml/kg) and was kept constant in each experiment. The jugular vein and carotid artery were cannulated for intravenous injections and for arterial blood pressure measurement with a pressure transducer (Statham P23AA, respectively). A catheter for measuring intrapleural pressure was inserted into the right intrapleural space between the fifth and sixth ribs via a surgical incision that was subsequently sutured and further sealed airtight with silicone jelly. The pneumothorax produced by this procedure was corrected by a brief opening of the intrapleural catheter to the ambient air during a held hyperinflation (3 × VT). The animals were paralyzed with an intravenous injection of pancuronium bromide (30 µg/kg) during the experiment to prevent the animals’ spontaneous breathing. Each time when the effect of pancuronium bromide wore off (~ 1 h) and before additional pancuronium bromide was given, the depth of anesthesia was checked and additional doses of anesthetics, if necessary, were administered to abolish the pain reflex. A heating pad was placed under the animal, which was lying in a supine position, to maintain the body temperature at 36–37°C during the experiment.

Transpulmonary pressure (Ptp) was measured as the difference between the tracheal pressure (Ptr) and the intrapleural pressure with a differential pressure transducer (Validyne MP-45-28, range ±50 cmH2O) positioned between a side arm of the tracheal cannula and the intrapleural cannula. Respiratory flow was measured with a pneumotachograph and a differential pressure transducer (Validyne MP-45-14, range ±2 cmH2O) and was integrated to give Vt; the pneumotachograph had a linear flow-pressure relationship in the range of 0–20 ml/s and a flow resistance of 0.046 cmH2O·ml−1·s−1. All signals were recorded on a chart recorder (Grass model 7), and total pulmonary resistance (RL) and dynamic lung compli-
challenges has already been described in a previous study (9). (10 mg/kg iv), a nicotinic acetylcholine-receptor antagonist. From HN cigarettes (HN-WS) were compared and PANAMINES as described above. Ten milliliters of 0.1 M phosphate buffer (pH 7.25) containing 10 µg/ml of meclofenamate, a cyclooxygenase inhibitor, were injected into the lungs via the tracheal cannula in each animal, withdrawn immediately, and reinjected; after the procedure was repeated three times, the recovered BALF was filtered through surgical gauze to remove the mucosa, centrifuged to remove cells, and stored at −80°C until the assay. Concentrations of prostanoids in the BALF were measured with the enzyme immunoassay method (Vmax kinetic microplate reader, Molecular Devices). Because PGE₂ is rapidly converted in the lungs to its 13,14-dihydro-15-keto metabolite that is unstable and undergoes further degradation to PGA products, these PGE₂ metabolites were first converted to stable bicyclo-PGE₂ before the assay. For a similar reason, PGD₂ was first converted to a stable methoxime (MOX) derivative (PGD₂-MOX) before the assay. All samples were tested in duplicate. Results are expressed in picograms per milliliter of BALF; the detection limits for bicyclo-PGE₂, TxB₂, PGD₂-MOX, and PGF₂α in BALF collected from both control animals (n = 6) and those challenged with 10 ml of LN-WS (n = 6) were measured; in the latter group, BALF was obtained −1.5 min after the smoke challenge when the Ptr reached its plateau.

Materials

A mixture of 2% α-chloralose (Sigma Chemical) and 10% urethan (Sigma Chemical) was dissolved in a 2% borax (Sigma Chemical) solution. Pancuronium bromide (2 mg/ml; Elkins-Sinn Pharmaceuticals) was diluted in saline. Hexamethonium bromide (Sigma Chemical) was dissolved in saline. SQ-29548 (Cayman Chemical) was dissolved in ethanol and diluted with 0.02% Na₂CO₃; this solution was further diluted in saline on the day of use to a concentration of 0.33 mg/ml (ethanol-0.02% Na₂CO₃:saline = 0.5:9.5:20, vol/ vol). The enzyme immunoassay kits were purchased from Cayman Chemical.

Statistical Analysis

Unless otherwise mentioned, a two-way analysis of variance (ANOVA) was used for the statistical analysis of data. One factor of the two-way ANOVA was the effect of smoke challenge in all study series; the other factor was the effect of hexamethonium in study series 1, effect of removing particulate matter in study series 2, effect of the number of challenges in study series 3, and effect of SQ 29548 in study series 4. When the two-way ANOVA showed a significant interaction, pairwise comparisons were made with a post hoc analysis (Fisher’s least significant difference test). In each animal, we averaged the six consecutive breaths immediately before the smoke challenge as the baseline, the six consecutive breaths with the maximum increase in RL that occurred within the first 20 breaths after the smoke inhalation as the first-phase response, and breaths 75–80 (last six breaths in Figs. 1–4) as the second-phase response.

RESULTS

Study Series 1: Effect of Hexamethonium on Bronchoconstriction Induced by HN-WS

Inhalation of 10 ml of HN-WS induced an immediate increase in Rl from a baseline of 0.22 ± 0.03 cmH₂O·ml⁻¹·s to a peak of 1.69 ± 0.35 cmH₂O·ml⁻¹·s in 10–15 breaths (P < 0.01; Fig. 1, Table 1). Rl then gradually declined toward the baseline but remained moderately elevated for a sustained period of time (1.5 min), although it did not reach a significant level (Fig. 1, Table 1). Cdyn decreased from a baseline of 0.48 ± 0.05 to 0.19 ± 0.05 ml/cmH₂O (P < 0.01) within 10–15 breaths after the smoke challenge and remained significantly lower than the baseline (P < 0.01) for the rest of the recording period (Table 1, Fig. 1). These
responses were similar to those described in a previous study (9) and could be divided into two distinct phases after appropriate pharmacological interventions: the first phase reached a peak rapidly within 20 breaths after the smoke inhalation and the second-phase response developed slowly and reached its plateau at ~1.5 min after the smoke challenge. Pretreatment with hexamethonium did not change the baselines of either RL or Cdyn, but it significantly reduced the first-phase response in RL and Cdyn by 86% (P < 0.01) and 50% (P < 0.01), respectively (Fig. 1). However, hexamethonium pretreatment did not significantly affect the second-phase response of either RL or Cdyn (Fig. 1, Table 1).

Cigarette smoke also induced a distinct cardiovascular response. The mean arterial blood pressure (MABP) first decreased transiently from a baseline of 55 ± 4 to 36 ± 2 mmHg (P < 0.01), concomitant with the first-phase response in RL and Cdyn. MABP then returned toward the baseline before a more sustained decrease (35 ± 4 mmHg; P < 0.01) slowly developed. After the smoke challenge, heart rate (HR) did not change initially but then gradually decreased from a baseline of 286 ± 6 beats/min to a steady state of 243 ± 8 beats/min after ~40 breaths (P < 0.01). Pretreatment with hexamethonium significantly reduced the baseline MABP to 30 ± 3 mmHg (P < 0.01) and decreased the baseline HR to 216 ± 6 beats/min (P < 0.01); furthermore, it completely abolished the changes in MABP and HR after the smoke challenge (Fig. 1).

Study Series 2: Effect of Removing Particulates from Cigarette Smoke on Bronchoconstriction

In sharp contrast to the response induced by HN-WS, the same volume of GPS did not elicit any detectable changes in RL or Cdyn during either the first or second phase (Table 1, Fig. 2). Similarly, GPS challenge did not cause significant changes in MABP or HR.

Study Series 3: Bronchoconstriction Induced by LN-WS

Inhalation of LN-WS induced a single delayed bronchoconstriction without any detectable first-phase response (Fig. 3). The time course of this delayed response was very similar to the second-phase response to HN-WS (9): beginning 15–25 breaths after the smoke inhalation, RL gradually increased and Cdyn gradually decreased from their baselines (RL, 0.16 ± 0.01 cmH2O·mL⁻¹·s; Cdyn, 0.64 ± 0.05 mL/cmH2O) and reached a steady state (RL, 0.37 ± 0.02 cmH2O·mL⁻¹·s; P < 0.01; Cdyn, 0.20 ± 0.04 mL/cmH2O, P < 0.01) ~50 breaths after the challenge (Fig. 3). Surprisingly, the response to the second LN-WS challenge was consistently smaller than that to the first one in all five animals tested (Fig. 3). However, the responses of RL (0.22 ± 0.02 cmH2O·mL⁻¹·s) and Cdyn (0.35 ± 0.06 mL/cmH2O) to the second challenge were still significantly higher than the baseline RL (0.15 ± 0.01 cmH2O·mL⁻¹·s; P < 0.05) and lower than the baseline Cdyn (0.58 ± 0.06 mL/cmH2O; P < 0.01), respectively. In addition, a third LN-WS challenge was given in four of these animals, and the response was not significantly different from that to the second one.

Study Series 4: Effect of SQ-29548 on Bronchoconstriction Induced by LN-WS

In the control group, LN-WS induced a delayed bronchoconstriction (Fig. 4). In the animals receiving the pretreatment with SQ-29548, the delayed bronchoconstriction induced by LN-WS was completely prevented; the RL
and $C_{dyn}$ 1.5 min after the smoke challenge were not significantly different from the baseline $R_L$ and $C_{dyn}$ (Fig. 4, Table 1). In the two animals pretreated with the vehicle solution for SQ-29548, the responses of $R_L$ and $C_{dyn}$ to LN-WS were similar to those obtained in the control group. To determine whether lung atelectasis was involved in the second-phase response, we tested the effect of a held hyperinflation ($4\times V_t$ for $5\ s$) on $R_L$ and $C_{dyn}$ in four additional animals. When the delayed response induced by LN-WS reached its plateau [change in ($\Delta$) $R_L$ = $118\pm 2\%$ of the baseline, $P < 0.05$; $\Delta C_{dyn} = -74\pm 3\%$, $P < 0.05$, one-way ANOVA] ~1.5 min after the smoke challenge, hyperinflation of the lungs twice effectively reversed the bronchoconstriction (e.g., Fig. 5); $\Delta R_L = 31\pm 3\%$ of the baseline, $P > 0.05$; $\Delta C_{dyn} = -9\pm 2\%$, $P > 0.05$ (one-way ANOVA).

Study Series 5: Effect of LN-WS on Prostanoid Levels in BALF

The concentration of TxB$_2$ in the BALF of the control group was $75.7\pm 22.2\ pg/ml$; it was elevated to

Table 1. Summary of responses in $R_L$ and $C_{dyn}$ in study series 1, 2, and 4

<table>
<thead>
<tr>
<th>Study series</th>
<th>$R_L$, cmH$_2$O·ml$^{-1}$·s</th>
<th>$C_{dyn}$, ml/cmH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 1st Phase 2nd Phase</td>
<td>Baseline 1st Phase 2nd Phase</td>
</tr>
<tr>
<td>Study series 1</td>
<td>0.22 $\pm$ 0.03 1.69 $\pm$ 0.35* 0.48 $\pm$ 0.08</td>
<td>0.48 $\pm$ 0.05 0.19 $\pm$ 0.05* 0.24 $\pm$ 0.03*</td>
</tr>
<tr>
<td>HN-WS</td>
<td>0.20 $\pm$ 0.01 0.41 $\pm$ 0.06† 0.58 $\pm$ 0.08</td>
<td>0.45 $\pm$ 0.04 0.33 $\pm$ 0.03† 0.24 $\pm$ 0.03*</td>
</tr>
<tr>
<td>H $+$ HN-WS</td>
<td>0.24 $\pm$ 0.01 1.33 $\pm$ 0.36* 0.38 $\pm$ 0.04</td>
<td>0.51 $\pm$ 0.04 0.26 $\pm$ 0.09* 0.28 $\pm$ 0.06*</td>
</tr>
<tr>
<td>Study series 2</td>
<td>0.23 $\pm$ 0.02 0.25 $\pm$ 0.03† 0.27 $\pm$ 0.04</td>
<td>0.57 $\pm$ 0.09 0.58 $\pm$ 0.09† 0.55 $\pm$ 0.09†</td>
</tr>
<tr>
<td>HN-WS</td>
<td>0.18 $\pm$ 0.01 NA 0.39 $\pm$ 0.03*</td>
<td>0.67 $\pm$ 0.05 NA 0.26 $\pm$ 0.03*</td>
</tr>
<tr>
<td>Study series 4</td>
<td>0.16 $\pm$ 0.02 NA 0.18 $\pm$ 0.02†</td>
<td>0.66 $\pm$ 0.05 NA 0.60 $\pm$ 0.05†</td>
</tr>
<tr>
<td>LN-WS</td>
<td>0.20 $\pm$ 0.01 0.41 $\pm$ 0.06† 0.58 $\pm$ 0.08</td>
<td>0.45 $\pm$ 0.04 0.33 $\pm$ 0.03† 0.24 $\pm$ 0.03*</td>
</tr>
<tr>
<td>SQ $+$ LN-WS</td>
<td>0.23 $\pm$ 0.02 0.25 $\pm$ 0.03† 0.27 $\pm$ 0.04</td>
<td>0.57 $\pm$ 0.09 0.58 $\pm$ 0.09† 0.55 $\pm$ 0.09†</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE; $n$, no. of animals. $R_L$, total pulmonary resistance; $C_{dyn}$, dynamic lung compliance; HN-WS, whole smoke generated from high-nicotine cigarettes; H, hexamethonium; GPS, gas-phase smoke generated from high-nicotine cigarettes; LN-WS, whole smoke generated from low-nicotine cigarettes; SQ, SQ-29548; NA, not applicable. Significant difference ($P < 0.01$) between: * baseline and response; † before and after treatment.
The concentration of PGD$_2$ was below the detection limit (<10 pg/ml) in five of the six control animals and 67.8 pg/ml in the remaining one; it was significantly higher in animals challenged with LN-WS (120.0 ± 6.8 pg/ml; P < 0.01, Wilcoxon rank sum test). The concentration of PGF$_2a$ was undetectable (<30 pg/ml) in all six control animals and 61.4 ± 7.0 pg/ml in the smoke group (P < 0.01, Wilcoxon rank sum test). The concentration of PGE$_2$ was 12.2 ± 3.9 pg/ml in the control group, and no significant difference was found in the smoke group (18.5 ± 2.2 pg/ml; P > 0.05, t-test).

**DISCUSSION**

Constituents in cigarette smoke can be classified into two major categories based on their physical nature: those in the particulate phase (including nicotine) and those in the gas phase. Previous studies have suggested that nicotine is the major bronchoconstrictive agent in cigarette smoke that causes immediate bronchoconstriction in dogs (8, 11, 14). However, the relative role of nicotine in the cigarette smoke-induced first- and second-phase bronchoconstriction in guinea pigs (9) was not known. Although hexamethonium, a nicotinic acetylcholine-receptor antagonist, prevented the first-phase bronchoconstrictive response, it had no effect on the second phase (Fig. 1, Table 1), indicating that nicotine was only responsible for evoking the first-phase response. This finding is not surprising because a previous study (9) has shown that the first-phase response to cigarette smoke is mediated by a combination of cholinergic reflex and tachykinin release; both of these mechanisms are presumably evoked by activation of bronchopulmonary C-fiber endings, and nicotine is known to be a potent stimulant of these afferent endings (11, 14). The previous finding that the stimulatory effect of nicotine on bronchopulmonary C-fiber endings is also blocked by pretreatment with hexamethonium lends additional support to this conclusion (14).

Results of the present study further suggest that the nonnicotine particulate matter is the primary causative agent producing the second-phase response to cigarette smoke because the response persisted even after hexamethonium pretreatment (Fig. 1, Table 1), whereas GPS failed to evoke any detectable effect (Fig. 2, Table 1). Additionally, LN-WS did not elicit any measurable first-phase bronchoconstriction but induced a clear second-phase response (Fig. 3, Table 1). The particulate matter of cigarette smoke consists of thousands of identifiable chemical components, and the specific causative agent(s) cannot be identified in this study.
It has been demonstrated that the second-phase bronchoconstrictive response to smoke persists even after the first-phase bronchoconstriction is completely blocked by a combination of atropine and tachykinin antagonists (9), suggesting that the second-phase response is not caused by the recirculation of the same agents that evoke the first-phase response (12). Furthermore, the second-phase response is completely inhibited by indomethacin pretreatment (9), indicating a role of cyclooxygenase metabolites. Indeed, the levels of several prostanoids (e.g., TxA2 and PGF2α) and their metabolites in BALF and/or serum have been shown to increase after inhalation of a larger volume of cigarette smoke (24, 25, 29). In addition, two types of prostanoid receptors, TP receptors and EP1 receptors that are involved in prostanoid-induced bronchoconstriction, have been identified in guinea pig airways (3, 18). Therefore, the exact types of prostanoids and their receptors mediating the second-phase bronchoconstriction remain to be determined. SQ-29548 is a potent and selective TP-receptor antagonist and does not block PGF2α-induced tracheal constriction in guinea pigs (21). Our results demonstrated that the second-phase response to LN-WS was completely prevented by pretreatment with SQ-29548 (Fig. 4, Table 1), indicating that TP-receptor activation was responsible for the second-phase bronchoconstriction. Interestingly, the TP receptor is also the primary receptor mediating prostanoid-induced constriction of human airways (1). Although TP receptors are more sensitive to TxA2, as is demonstrated by its higher sensitivity to U-46619, a TxA2 mimetic, than to other prostanoids, some other prostanoids such as PGD2 and PGF2α may also activate TP receptors to some extent and induce airway smooth muscle contraction (21, 26). Therefore, we assayed four naturally occurring prostanoids or their metabolites in the BALF of both control animals and animals challenged with LN-WS to determine the types of prostanoids that may have a role in the activation of TP receptors after smoke challenge. Although our data showed that the concentrations of all three major bronchoconstrictive prostanoids (TxA2, PGD2, and PGF2α) increased after smoke challenge, the much higher increase in TxB2 in the BALF in conjunction with the high sensitivity of TP receptors to TxA2 suggests an important role of TxA2 in mediating the second-phase response to cigarette smoke. Several prostanoids, including TxA2 and PGF2α, have been shown to increase pulmonary vascular pressure, vascular permeability, and transvascular filtration rate in the lungs, which may then lead to pulmonary edema and reduced lung compliance (17). However, the fact that hyperinflation of the lungs at the plateau of the second-phase response immediately reversed the increased RL and the decreased Cdyn (Fig. 5) suggests that constriction and/or closure of small airways, followed by regional atelectasis instead of lung edema, is the primary cause of the observed changes.

The mechanism underlying the attenuated response to the second LN-WS challenge is not completely understood. U-46619 (2 µg/kg iv; Cayman Chemical), a selective TP-receptor agonist, evoked bronchoconstriction at an intensity similar to that caused by LN-WS but did not cause any tachyphylaxis in two consecutive challenges in three animals tested (Hong and Lee, unpublished observations). Thus a reduced sensitivity of TP receptors of airway smooth muscles during the second smoke challenge cannot account for the tachyphylaxis. It seems plausible that the difference between the two responses may be due to a reduced synthesis of prostanoids. It has been reported that nicotine in vitro selectively inhibits TxA2 synthesis in macrophage-like cells (6), platelets, and lung tissues (25); whether the first cigarette smoke inhalation challenge inhibited TxA2 synthase and led to a smaller amount of TxA2 being released in response to the second challenge is not known. Alternatively, substrate-induced (suicide) inactivation of cyclooxygenase (27) and Tx synthase (10) may also play a role in the observed tachyphylaxis. Coincidently, when the systemic infusion of acid solution that triggered the release of TxA2 from platelets was repeated in 1.5-h intervals in anesthetized cats, a smaller amount of TxA2 was released and, consequently, less intense pulmonary hypertensive and hyperventilatory responses were elicited during the second and third challenges (23).

In addition to bronchoconstriction, cardiovascular changes in the reflex response elicited by activation of pulmonary C fibers include transient hypotension and bradycardia in dogs, cats, and rats (4). In anesthetized spontaneous-breathing guinea pigs, however, stimulation of pulmonary C fibers by intravenous capsaicin, a selective C-fiber stimulant, elicits hypotension without significant changes in HR (22). Similar cardiovascular responses (transient hypotension without accompanying bradycardia) were also observed immediately after the HN-WS inhalation into the airways of anesthetized and artificially ventilated guinea pigs in this study, coinciding with the first-phase bronchoconstriction (Fig. 1) that stems partially from vagal cholinergic reflex (9). The fact that pretreatment with hexamethonium completely prevented the transient hypotension seems to support the notion that nicotine in the cigarette smoke is responsible for activating bronchopulmonary C fibers (11, 13). Alternatively, because systemic administration of hexamethonium also blocks the ganglionic transmission of the autonomic nervous system, the hypotensive response elicited by activating other afferents or evoked by smoke constituents other than nicotine will also be blocked. The mechanisms underlying the delayed and prolonged hypotension and bradycardia induced by cigarette smoke could not be determined in our study. Activation of bronchopulmonary C fibers by cigarette smoke triggers the release of calcitonin gene-related peptide (15), a potent vasodilator, which is colocalized in and coreleased from these endings with tachykinins (16). Furthermore, a direct negative inotropic effect of nicotine on the guinea pig heart has been reported, and this effect is also blocked by hexamethonium (2).

In conclusion, our present study demonstrates that nicotine is the primary agent responsible for triggering
the first-phase bronchoconstrictive response to cigarette smoke, whereas nonnicotine smoke particulates play a major role in the second-phase response in guinea pigs. Additionally, these results suggest that smoke particulates induce the second-phase response by releasing bronchoconstrictive prostanoids TXA\(_2\), PGD\(_2\), and PGF\(_2\) that act on TP receptors of airway smooth muscles and cause a constriction and/or closure of peripheral airways.

The authors are grateful to Dr. Hsin-Hsiung Tai for helpful advice and to Dr. Timothy McClintock for making the Vmax kinetic microplate reader available for the enzyme immunoassay experiment. The authors also thank Dr. Mary K. Rayens for statistical consultation and Robert Morton for technical assistance.

This study was supported by National Heart, Lung, and Blood Institute Grants HL-40369 and HL-52172 and by Grant 5-R01-HL-2686 from the Kentuck Tobacco and Health Research Board.

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Received 14 December 1995; accepted in final form 12 July 1996.

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