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**DIFFERENCE IN THE EFFECTS OF
CIGARETTE SMOKE EXPOSURE ON
BRONCHIAL SMOOTH MUSCLE
CONTRACTILITY IN RATS**YOSHIHIKO CHIBA*, MASAHIKO MURATA, YUJI YOSHIKAWA,
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PHARMACY, HOSHI UNIVERSITY, TOKYO, JAPAN (Y.Y., A.S., J.K.)**RESEARCH ARTICLE**

ABSTRACT. CIGARETTE SMOKING is a risk factor for development of airway hyperresponsiveness and chronic obstructive pulmonary disease (COPD). However, little is known concerning the effect of cigarette smoking on the contractility of airway smooth muscle. The current study was carried out to determine the responsiveness of bronchial smooth muscles isolated from rats that were chronically exposed to mainstream cigarette smoke (CS) *in vivo*. The bronchial smooth muscle responsiveness was compared in three strains of rats. Male Wistar, Brown-Norway (BN) and Sprague-Dawley (SD) rats were exposed to diluted mainstream CS for 2 hours/day, every day for 2 weeks. Twenty-four hours after the last CS exposure, the smooth muscle responsiveness of isolated left main bronchus was measured. The concentration-response curve to acetylcholine (ACh) of bronchial smooth muscle isolated from the CS-exposed group was significantly shifted upward as compared with that from the air-exposed control in Wistar rats, but not in BN and SD strains. In none of the strains of rats, significant change in response to high K⁺-depolarization of bronchial smooth muscle was observed after the chronic CS exposure. In conclusion, *in vivo* exposure to CS caused a significant bronchial smooth muscle hyperresponsiveness to ACh in Wistar rats, but not in BN and SD strains. The Wistar strain of rats might be useful for studying and understanding the mechanism of bronchial hyperresponsiveness induced by cigarette smoking.

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1. INTRODUCTION

Cigarette smoking is the major cause of chronic obstructive pulmonary disease (COPD), which is one of the most important causes of morbidity and mortality in the world. Cigarette smoking induces an inflammatory response in the airways that might play a key role in the pathogenesis of COPD. Furthermore, multicenter clinical trials (Lung Health Study) showed that current smokers with functional evidence of early COPD have airway hyperresponsiveness [1,2]. Similarly, a dose-dependent effect of cigarette smoking on airway responsiveness has also reported [3]. The latter study would support the concept that cigarette smoke (CS) has a primary effect on airway responsiveness.

Experimental evidence has been reported that chronic exposure to CS augments the *in vivo* responsiveness of airways to cholinergic agonists in rodents [4-6]. These findings suggest that cigarette smoking causes airway hyperresponsiveness although the underlying mechanism is not fully understood. CS contains many chemical and oxidizing pollutants, some of which have been reported to affect airway smooth muscle contractility directly [7,8]. One possible mechanism of the CS-induced airway hyperresponsiveness may be a change in airway smooth muscle contractility as reported in animal models of allergic bronchial asthma [9-11]. Rapid relief from airway limitation in patients with COPD by bronchodilators, such as an anti-cholinergic tiotropium bromide [12,13], may also support the hypothesis. However, little is known concerning the effect of cigarette smoking *in vivo* on the contractility of airway smooth muscle *in vitro*. On the other hand, some strain-related difference in the response to CS has been reported in the murine models [14-16]. In the present study, the effect of chronic CS exposure on bronchial smooth muscle responsiveness was compared in three strains of rats, *i.e.*, Wistar, Brown-Norway (BN) and Sprague-Dawley (SD) rats.

2. MATERIAL AND METHODS

2.1. ANIMALS AND CIGARETTE SMOKE

EXPOSURE

Male Wistar, Brown-Norway (BN) and Sprague-Dawley (SD) rats (6 weeks of age, Charles River Japan, Inc., Kanagawa, Japan) were used. All experiments were approved by the Animal Care Committee at Hoshi University (Tokyo, Japan).

Each strain of rats was randomly divided into two groups to be exposed to either mainstream cigarette smoke (CS group) or room air (control group). In the CS group, animals were exposed to diluted mainstream cigarette smoke for 2 hours/day, every day for 2 weeks, by using an automated smoking machine (Model INH06-CIGR01; Medical Interface Project Station, Inc., Osaka, Japan). In brief, each awake rat was held in an exposure chamber, which was connected with the smoking machine. A puff of mainstream cigarette smoke (35 mL) generated from *hi-lite*TM cigarettes (a total of 1.4 mg nicotine and 17 mg tar/cigarette; Japan Tobacco, Inc., Tokyo, Japan) was diluted with 280 mL of room air and then delivered to the chamber. Each cigarette was puffed forty times with suction volume of 600 mL/min.

2.2. FUNCTIONAL STUDY OF BRONCHIAL SMOOTH MUSCLES

To determine the effect of cigarette smoke exposure *in vivo* on the bronchial smooth muscle responsiveness *in vitro*, the isometrical contraction of the circular smooth muscle of the main bronchus was measured as described previously [17,18]. In brief, 24 hours after the last cigarette smoke or room air exposure, the rats were sacrificed by exsanguinations from abdominal aorta under chloral hydrate (400 mg/kg, *i.p.*) anesthesia. Then the airway tissues below the larynx to lungs were immediately removed. About 4 mm length (3 mm diameter) of the left main bronchus was isolated (8-9 cartilages), and the resultant tissue ring preparation was then suspended in an organ bath at a resting tension of 1 g. The organ bath contained modified Krebs-Henseleit solution with the following composition (mM); NaCl 118.0, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2 and glucose 10.0 (pH 7.4). The isometrical contraction of the circular smooth muscle was

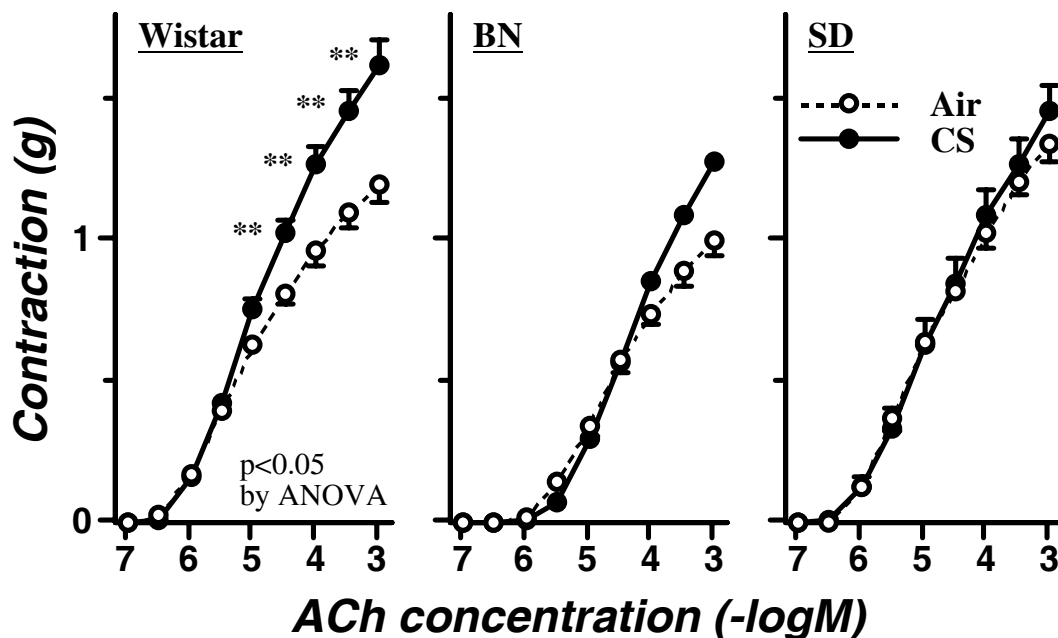


FIGURE 1. EFFECT OF REPEATED EXPOSURE TO MAINSTREAM CIGARETTE SMOKE (CS) ON BRONCHIAL SMOOTH MUSCLE RESPONSIVENESS TO ACh IN WISTAR (LEFT), BROWN-NORWAY (BN; MIDDLE) AND SPRAGUE-DAWLEY (SD; RIGHT) RATS. Smooth muscle ring preparations (about 4 mm length, 3 mm diameter) were isolated from the left main bronchi of the 2-week CS- (closed circles) or room air-exposed animals (Air; open circles). Each point represents the mean \pm SEM from 8-15 animals. Note that the concentration-response curve to ACh of bronchial smooth muscle was significantly shifted upward only in the CS group of Wistar rats ($p < 0.05$ by two-way ANOVA). ** $P < 0.01$ vs. respective air group by unpaired Student's t -test.

measured with a force-displacement transducer (TB-612T, Nihon Kohden, Tokyo, Japan). During an equilibration period, the tissues were washed three or four times at 15–20-min intervals and were equilibrated slowly to a baseline tension of 1 g. After the equilibration period, the concentration-response curve to acetylcholine (ACh; 10^{-7} – 10^{-3} M in final concentration) was constructed cumulatively. A higher concentration of ACh was successively added after attainment of a plateau response to the previous concentration. In another series of experiment, isotonic K^+ solution (10–90 mM in final concentration) was cumulatively administered in the presence of atropine and indomethacin (both 10^{-6} M) to determine the bronchial smooth muscle responsiveness to high K^+ -depolarization.

2.3. STATISTICAL ANALYSES

All the data were expressed as the mean \pm S.E.

Statistical significance of the difference was determined by using unpaired Student's t -test or two-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered significant.

3. RESULTS

To determine the effect of chronic exposure to mainstream cigarette smoke (CS) on bronchial smooth muscle contractility, the smooth muscle responsiveness to stimulation of plasma membrane receptors, such as muscarinic cholinceptors, and non-receptor-mediated stimulation, *i.e.*, isotonic high K^+ -depolarization, were determined.

As shown in FIG. 1, acetylcholine (ACh) stimulation caused concentration-dependent contractile responses both in the air-treated control and CS-exposed groups in the three strains of rats. The contraction induced by ACh was inhibited by

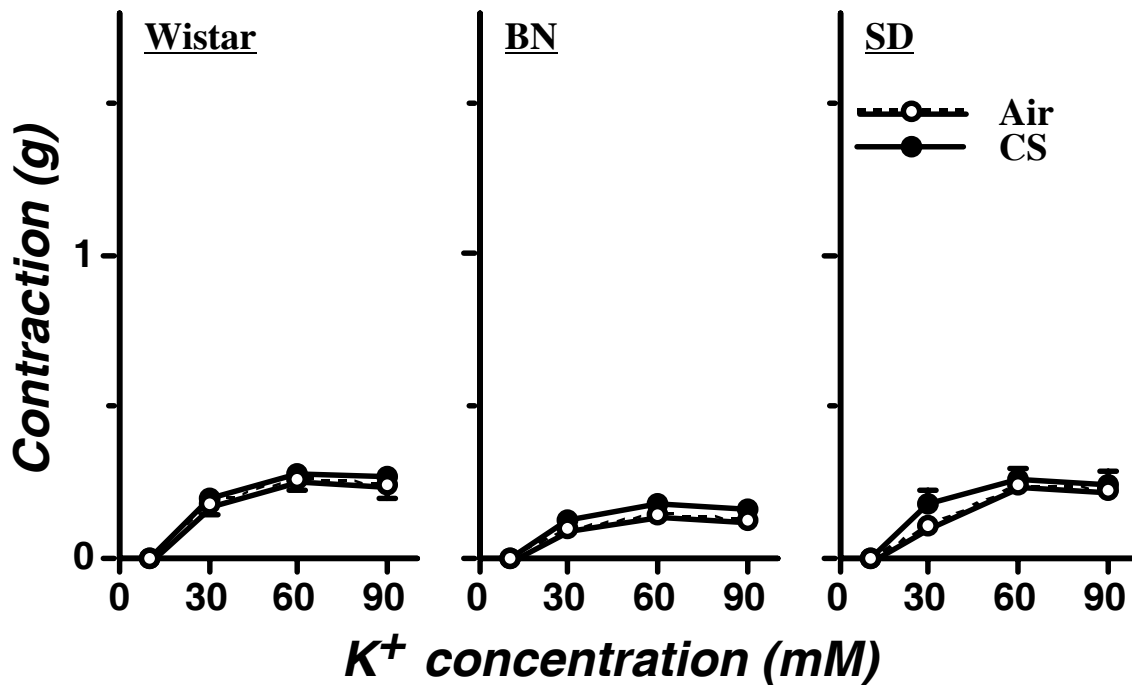


FIGURE 2. EFFECT OF REPEATED EXPOSURE TO MAINSTREAM CIGARETTE SMOKE (CS) ON BRONCHIAL SMOOTH MUSCLE RESPONSIVENESS TO ISOTONIC HIGH K^+ -DEPOLARIZATION IN WISTAR (LEFT), BROWN-NORWAY (BN; MIDDLE) AND SPRAGUE-DAWLEY (SD; RIGHT) RATS. Smooth muscle ring preparations (about 4 mm length, 3 mm diameter) were isolated from the left main bronchi of the 2-week CS- (closed circles) or room air-exposed animals (Air; open circles). The smooth muscle responsiveness to high K^+ -depolarization was measured in the presence of atropine and indomethacin (both 10^{-6} M). Each point represents the mean \pm SEM from 8-15 animals. Note that no significant difference in high K^+ responsiveness was observed between CS and Air groups in any strains of rats.

atropine (10^{-6} M; data not shown) completely and by 4-diphenylacetoxy N-methylpiperidine, an M_3 receptor antagonist, with high affinity [19], indicating an involvement of M_3 subtype of receptors in the ACh-induced contraction of rat bronchial smooth muscle.

In Wistar strain of rats, the ACh-induced contraction of bronchial smooth muscles isolated from the CS-exposed group was significantly augmented as compared with that from the air-treated control animals. The concentration-response curve to ACh of bronchial smooth muscle isolated from the CS-exposed group was significantly shifted upward ($P < 0.05$ by two-way ANOVA; FIG. 1, left panel). Although no change in the pD_2 value (-logarithm of 50% effective ACh concentration (M), calculated by individual concentration-response curve) was observed

between groups (5.05 ± 0.06 in the air-treated control and 4.92 ± 0.08 in the CS-exposed group), the maximal contraction (E_{MAX}) was significantly increased in the CS-exposed animals (1.63 ± 0.09 g) when compared with the control group (1.20 ± 0.08 g; $P < 0.01$). On the other hand, no significant difference in the contractile response induced by high K^+ -depolarization was observed between groups (FIG. 2, left panel).

In Brown-Norway (BN) rats, a significant increase in the E_{MAX} of ACh-induced contraction of bronchial smooth muscles from the CS-exposed animals (1.28 ± 0.02 g) was observed as compared with that from the air-treated control group (1.00 ± 0.05 g; $P < 0.001$; FIG. 1, middle panel). However, the pD_2 value of the CS-exposed group (4.39 ± 0.06) was significantly decreased rather than increased as compared with that of the control one

(4.65 ± 0.04 ; $P < 0.01$). When the ACh concentration-response curves were compared, no significant difference was obtained between the groups ($P > 0.05$ by ANOVA). As well as the Wistar strain, the bronchial smooth muscle responsiveness to high K^+ -depolarization of the CS-exposed animals was control level (FIG. 2, middle panel).

In case of Sprague-Dawley (SD) rats, CS-exposure did not affect the bronchial smooth muscle responsiveness to ACh (FIG. 1, right panel) and high K^+ (FIG. 2, right panel).

4. DISCUSSION

The current study demonstrated that the 2-week exposure of mainstream cigarette smoke (CS) caused a marked hyperresponsiveness to ACh, but not to high K^+ -depolarization, of isolated bronchial smooth muscle in Wistar strain of rats. A moderate increase in the ACh response was also observed in BN rats, but no change was observed in SD strain. It is thus possible that the change in the bronchial smooth muscle responsiveness induced by *in vivo* exposure to CS might be strain-dependent in rats.

It has been suggested that cigarette smoking is a risk factor for development of airway hyperresponsiveness and COPD [1-3]. Experimental evidence has also demonstrated an augmented *in vivo* responsiveness of airways following CS exposure [4-6]. Although many investigators have made an effort to clarify the mechanism(s) of CS-induced airway hyperresponsiveness, the underlying mechanism is still unclear. Xu et al. [6] found that exposure to mainstream CS resulted in an *in vivo* airway hyperresponsiveness to methacholine in Long-Evans rats. In their study, however, there was no evidence that the hyperresponsiveness was related to changes in elastic recoil pressure of airways, to airway-parenchymal interdependence, or to airway inflammation [6], all of which have been reported to affect airway responsiveness *in vivo* [20-23]. Furthermore, some of the chemical and oxidizing pollutants generated by cigarette smoking affect airway smooth muscle contractility directly [7,8]. We thus hypothesized that airway hyperrespon-

siveness induced by CS exposure might result from an alteration in airway smooth muscle contractility. On the other hand, some strain-related difference in the response to CS has been reported in the murine models [14-16]. So in the present study, the effect of chronic CS exposure *in vivo* on bronchial smooth muscle responsiveness *in vitro* was compared among the three strains of rats.

In Wistar strain of rats, a marked augmentation of ACh responsiveness with statistical significance was observed in bronchial smooth muscle isolated from the CS-exposed animals, whereas the response to high K^+ -depolarization did not change. The results suggest that the receptor-mediated signaling rather than the Ca^{2+} influx from voltage-dependent Ca^{2+} channels might be augmented in bronchial smooth muscle of the CS-exposed Wistar rats. Furthermore, the Ca^{2+} responsiveness of contractile elements without agonist stimulation might be normal, because no change in the response to high K^+ -depolarization between groups was observed. Although the underlying mechanism(s) responsible for the CS-induced bronchial smooth muscle hyperresponsiveness is not known now, the pharmacological evidence, *i.e.*, significant increase in maximal ACh-induced contraction without change in the pD_2 value (Fig. 1, left panel), may suggest an upregulation of muscarinic cholinergic receptors, probably M_3 subtypes, in bronchial smooth muscle of the CS-exposed animals. Alternatively, our preliminary study showed an augmented ACh-induced Ca^{2+} sensitization of contraction in α -toxin-permeabilized bronchial smooth muscle of the CS-exposed Wistar rats. Further studies are needed for understanding the mechanism of bronchial smooth muscle hyperresponsiveness after chronic CS exposure observed in the Wistar strain of rats.

In contrast to the Wistar rats, chronic CS exposure *in vivo* had no effect on the responsiveness to ACh and high K^+ -depolarization of isolated bronchial smooth muscle in the SD strain of rats. The results might be consistent with the previous report. Wright et al. [24] showed that long-term exposure to CS (for 2, 4, 8 and 12 months, 7 cigarettes/day, 5 days each week) did not alter the airway responsiveness to methacholine in SD rats.

This strain of rats may be resistant to CS. Making clear the mechanism(s) of the difference in the pathogenesis of CS-induced bronchial smooth muscle hyperresponsiveness between Wistar and SD strains may provide us some information on why not all smokers fall into COPD.

The BN strain of rats is known as a high IgE responder and has frequently been used for a model of allergen-induced airway hyperresponsiveness [25-27]. The current study did not show any advantage of the BN strain as a model for CS-induced bronchial smooth muscle hyperresponsiveness when compared with Wistar rats. However, it is of interest to examine the effect of chronic CS exposure on antigen-induced airway hyperresponsiveness in the BN rats, because the contribution of CS to the prevalence and occurrence of exacerbations in allergic bronchial asthma has been suggested [28,29].

In conclusion, *in vivo* exposure to CS caused a significant bronchial smooth muscle hyperresponsiveness to ACh in Wistar rats, but not in BN and SD strains. The Wistar strain of rats might be useful for understanding the mechanism of bronchial hyperresponsiveness induced by cigarette smoking. Although the mechanism of the strain-related difference is not clear now, it may give us some answers why only a part of smokers suffer from COPD.

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