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Role of adrenergic nervous system in cigarette smoke-induced bronchoconstriction in guinea pigs

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Abstract

The goal of this study was to clarify the role of the adrenergic nervous system in bronchoconstriction induced by exposure to cigarette smoke in guinea pigs. Artificially ventilated animals were exposed to 160 puffs of smoke for 8 min. Bronchoconstriction was assessed as a percentage of the baseline total pulmonary resistance (R_L). The effects of pretreatment with phentolamine (0.1 mg/kg, i.v.), propranolol (1 mg/kg, i.v.), and/or atropine (1 mg/kg, i.v.) were evaluated. Exposure to cigarette smoke caused significant bronchoconstriction. Phentolamine, an α-adrenoceptor antagonist, significantly inhibited cigarette smoke-induced bronchoconstriction, while propranolol, a β-adrenoceptor antagonist, significantly enhanced it. Combined use of these compounds further enhanced the bronchoconstriction. All of modulations of the bronchoconstriction by adrenoceptor antagonists were completely abolished by pretreatment with atropine. Phentolamine and/or propranolol had no effect on the bronchoconstriction induced by inhaled acetylcholine. Pretreatment with yohimbine (0.5 mg/kg, i.v.), a selective α_2-adrenoceptor antagonist, showed modulatory effects similar to those of phentolamine on cigarette smoke-induced bronchoconstriction. These results suggest that cigarette smoke-induced bronchoconstriction is regulated by the prejunctional modulation of the cholinergic system via α- and β-adrenoceptors. This mechanism may be modulated by the autoregulation of adrenergic nerves via the α_2-autoreceptor.

Keywords: (Guinea pig); α-Adrenoceptor; β-Adrenoceptor; Cholinergic nerve

1. Introduction

Acute exposure to cigarette smoke provokes bronchoconstriction in guinea pigs. Previous studies have suggested that multiple mediators, including tachykinins, thromboxane, and acetylcholine are involved in the cigarette smoke-induced bronchoconstriction (Hong et al., 1995; Lee et al., 1995; Hong and Lee, 1996; Matsumoto et al., 1996a,b). However, little is known about the protective system against excessive bronchoconstriction induced by cigarette smoke.

The adrenergic nervous system is one of the crucial means for regulating bronchoconstriction in several mammalian species (Barnes, 1986). In guinea pig airways, abundant innervation of adrenergic nerves, and specific receptors, have been described (O'Donnell et al., 1978; Barnes et al., 1980; Gatto et al., 1984). Previous studies have reported that cholinergic contraction of airway smooth muscle was inhibited pre-junctionally by β_2-adrenoceptors located on cholinergic nerves and airway epitherium (Aizawa et al., 1991; Wessler et al., 1994). Cholinergic contraction has been shown to be pre-junctionally inhibited by α_2-adrenoceptors (Wikberg, 1978; Matran et al., 1989; Thompson et al., 1990). An important function of the α_2-adrenoceptor is the autoinhibition of noradrenaline release from adrenergic nerve termini (Hoffman and Lefkowitz, 1980; Starke et al., 1989). In contrast, stimulation of α_2-adrenoceptors on airway smooth muscles has been reported to cause muscle contraction in guinea pigs (Fliesh et al., 1970; Takayanagi et al., 1991). The majority of this evidence was obtained from in vitro experiments; their precise roles of α_2-adrenoceptors in vivo remain uncertain. Also, not much information exists concerning whether autonomic regulation of adrenergic nerves has a considerable role in cigarette smoke-induced bronchoconstriction.

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The aim of this study was to clarify the role of the adrenergic nervous system in bronchoconstriction induced by exposure to cigarette smoke. We evaluated the effects of an α-adrenoceptor antagonist, phentolamine, and a β-adrenoceptor antagonist, propranolol, on the bronchoconstriction. To elucidate its possible interaction with cholinergic mechanism, we further investigated the effect of atropine on cigarette smoke-induced bronchoconstriction, and also the effects of phentolamine and propranolol on the bronchoconstriction induced by inhaled acetylcholine.

2. Materials and methods

2.1. Study protocol

Hartley strain male guinea pigs weighing 500 to 600 g (Kyudo, Kumamoto, Japan) were anesthetized intraperitoneally with 50 mg/kg pentobarbital sodium. Animals were intubated via tracheostomy and mechanically ventilated with a respirator (Model 683, Harvard Apparatus, South Natick, MA, USA) at a constant tidal volume of 7 ml/kg and a rate of 60 breaths/min. A catheter was introduced into the jugular vein to administer drugs.

2.1.1. Protocol 1

Twenty-eight animals were randomly divided into 4 groups (7 per group): vehicle control (0.9% saline, 1 ml/kg); phentolamine (0.1 mg/kg); propranolol (1 mg/kg); and phentolamine and propranolol. After the baseline total pulmonary resistance (R_L) was determined, vehicle, phentolamine, propranolol, or both drugs were administered intravenously. The doses of phentolamine and propranolol were based on previous studies (Lei et al., 1995; Matsumoto et al., 1996b). Ten min after drug administration, the animals were exposed to cigarette smoke for 8 min. In processing this protocol, additional fourteen animals were used for examining the effects of yohimbine (0.5 mg/kg, i.v.), a selective α_2-adrenoceptor antagonist, on the cigarette smoke-induced response. The dose of yohimbine was based on a previous study (Grundstrom and Andersson, 1985).

2.1.2. Protocol 2

Twenty-eight animals were randomly divided into 4 groups (7 per group): atropine (1 mg/kg); phentolamine and atropine; propranolol and atropine; and phentolamine, propranolol and atropine. After the baseline R_L was determined, drugs were administered intravenously. The dose of atropine was based on previous studies (Lei et al., 1995; Matsumoto et al., 1996a,b). Ten min after, the animals were exposed to cigarette smoke for 8 min.

2.1.3. Protocol 3

Twenty animals were randomly divided into 4 groups (5 per group): vehicle control (0.9% saline, 1 ml/kg); phentolamine; propranolol; and phentolamine and propranolol. After the baseline R_L was determined, drugs were administered intravenously. Ten min after, the animals were exposed to increasing doses of inhaled acetylcholine as described below.

2.2. Measurement of total pulmonary resistance (R_L)

To estimate pleural pressure, a fluid-filled catheter was introduced into the esophagus at a point such that the maximal amplitude of pressure was obtained. The animals were placed supine in a flow-type body plethysmograph. The plethysmograph airflow was measured with a Fleisch pneumotachograph (model TV-132, Nihon Kohden, Tokyo, Japan) and a differential pressure transducer (model TP-602T, Nihon Kohden, Tokyo, Japan). The plethysmograph was made of Plexiglass and had 2.80 l of dead space (customized, Chest Medical, Tokyo, Japan). Transpulmonary pressure was estimated as the difference between the esophageal and airway opening pressures, measured by a differential pressure transducer (model TP-603T, Nihon Kohden, Tokyo, Japan). Total pulmonary resistance (R_L) was calculated from the transpulmonary pressure and plethysmograph airflow (Amdur and Mead, 1958).

2.3. Exposure of cigarette smoke

Exposure to cigarette smoke was achieved as described previously (Matsumoto et al., 1996a,b). Briefly, cigarette

Table 1
Baseline pulmonary resistance among groups

Protocol 1 (cigarette smoke)	control	phentolamine	propranolol	phentolamine and propranolol
R_L (cmH_2O/ml per s)	0.306 ± 0.007	0.313 ± 0.012	0.319 ± 0.009	0.331 ± 0.011
	N.S.			
Protocol 2 (cigarette smoke)	atropine	phentolamine and atropine	propranolol and atropine	phentolamine, propranolol and atropine
R_L (cmH_2O/ml per s)	0.290 ± 0.013	0.289 ± 0.013	0.329 ± 0.011	0.309 ± 0.019
	N.S.			
Protocol 3 (inhaled ACh)	control	phentolamine	propranolol	phentalomine and propranolol
R_L (cmH_2O/ml per s)	0.307 ± 0.019	0.298 ± 0.022	0.298 ± 0.018	0.288 ± 0.017
	N.S.			

Abbreviations: R_L, total pulmonary resistance; N.S., not significant; ACh, acetylcholine.

smoke was supplied by a smoke generator using a volume-controlled respirator (Model 681, Harvard Apparatus, South Natick, MA, USA) set at a constant tidal volume of 3.5 ml and a rate of 60 breaths/min. Smoke from a cigarette directly connected to the ventilation circuit was delivered to each animal in 10 consecutive breaths, followed by 20 breaths of fresh air. This procedure was repeated for 8 min. Thus, the animals were intermittently exposed to a total of 160 puffs of cigarette smoke over 8 min. In a preliminary study, we had confirmed that the resistance remained unchanged throughout the 8 min with sham exposure ($100 \pm 2\%$ of baseline R_L ($n = 7$)), and that this protocol for exposure did not produce serious hypoxemia or circulatory disturbances. Cigarettes were purchased from Japan Tobacco (Tokyo, Japan). Each cigarette contained 2.7 mg of nicotine and 26 mg of tar, according to the manufacturer's specifications.

2.4. Measurement of acetylcholine-induced bronchoconstriction

Bronchoconstriction to exogenously applied acetylcholine was determined by inhalation of an increasing concentration of agents administered via the endotracheal tube. The aerosols (output, 1.5 ml/min) were generated by an ultrasonic nebulizer (model TUR-3200, Nihon Kohden, Tokyo, Japan) placed in line with the ventilator. Dose–response curves were constructed as follows: saline was given for 15 breaths, and the subsequent R_L value was used as the baseline. The aerosol was administered for 15 breaths, separated by 5-min intervals. The concentration of bronchoconstrictor agents was increased for each series of 15 breaths. R_L was monitored for 5 min after each nebulization, and the maximum value was plotted against the agent concentration. To achieve a constant-volume history, hyperinflations (triplicate of tidal volume) were obtained between each agent challenge.

2.5. Materials

The drugs used in this study were atropine sulfate (Tanabe Pharmaceutical, Osaka, Japan), propranolol hydrochloride (Zeneca Pharmaceutical, Osaka, Japan), phentolamine mesylate (Ciba-Geigy, Takarazuka, Japan), and pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA). Yohimbine hydrochloride (Sigma, St. louis, MO, USA) was dissolved in acid saline (0.9% NaCl with HCl added to a pH of 2.6).

2.6. Statistical analysis

Data are expressed as the mean ± standard error of the mean (S.E.M.). Baseline R_L was compared among all groups by one-way analysis of variance (ANOVA). Values for R_L are expressed as the percentage of the baseline R_L. Time-course curves for R_L during the exposure to smoke between each drug-treated group and control group were assessed by two-way ANOVA, followed by Dunnett's tests. A level of $P < 0.05$ was accepted as statistically significant.

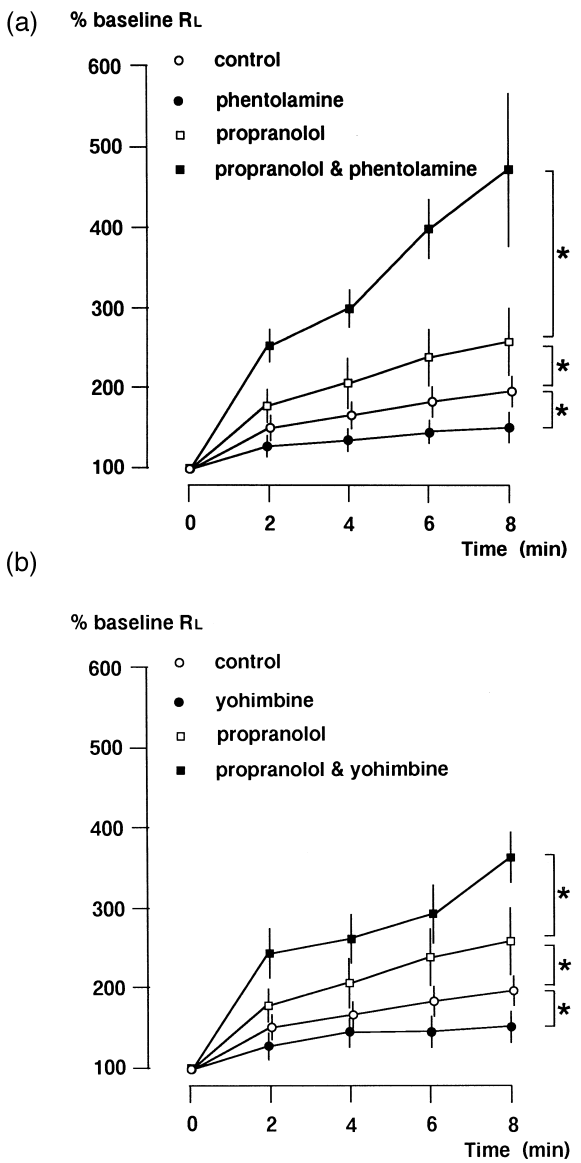

Fig. 1. Effect of adrenoceptor antagonists on cigarette smoke-induced bronchoconstriction. Pretreatment with phentolamine significantly inhibited cigarette smoke-induced bronchoconstriction, compared with the control vehicle-treated animals (a) Pretreatment with propranolol significantly enhanced bronchoconstriction compared with control. Combined use of phentolamine and propranolol significantly enhanced bronchoconstriction, compared with propranolol-treated animals. Pretreatment with yohimbine alone significantly inhibited bronchoconstriction (b). In contrast, pretreatment with yohimbine significantly enhanced bronchoconstriction in propranolol-treated animals. Values represent the mean ± S.E. for seven animals. $* P < 0.05$.

3. Results

3.1. Baseline pulmonary resistance among groups

There were no significant differences in baseline R_L values among the groups in each protocol (Table 1).

3.2. Effect of adrenoceptor antagonists on bronchoconstriction induced by cigarette smoke

Vehicle-treated, cigarette smoke-exposed animals exhibited time-dependent bronchoconstriction, as evidenced by increased R_L (Fig. 1a). Pretreatment with phentolamine significantly inhibited bronchoconstriction ($P < 0.05$). Conversely, pretreatment with propranolol significantly enhanced the bronchoconstriction ($P < 0.05$). Combined use of phentolamine and propranolol allowed for more bronchoconstriction than the propranolol-treated animals ($P < 0.05$). The effects of yohimbine were quite similar to those of phentolalmine (Fig. 1b). Thus, yohimbine alone significantly inhibited bronchoconstriction ($P < 0.05$), whereas yohimbine in combination with propranolol strongly enhanced bronchoconstriction induced by cigarette smoke ($P < 0.05$).

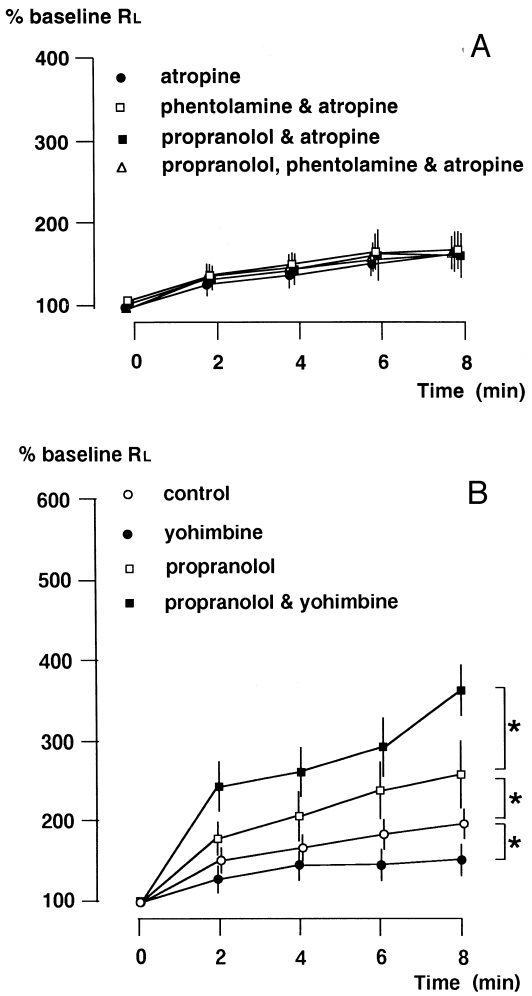

Fig. 2. Effect of atropine on the adrenoceptor antagonist-mediated modulations of bronchoconstriction induced by cigarette smoke. Modulations of cigarette smoke-induced bronchoconstriction by adrenergic antagonists were not observed in the animals pretreated with atropine. Values represent the mean ± S.E. for seven animals.

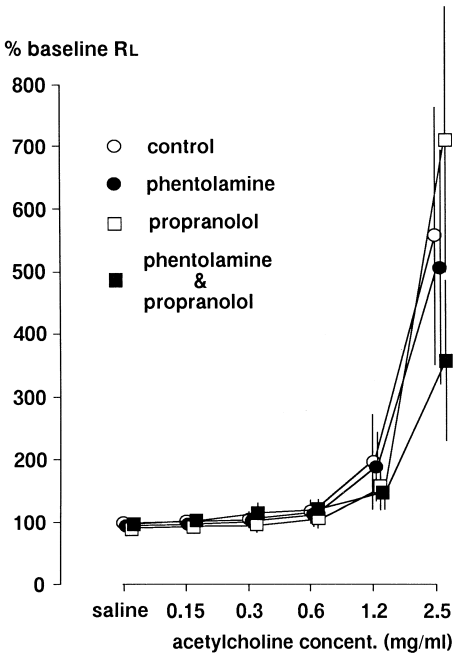

Fig. 3. Effect of adrenoceptor antagonists on inhaled acetylcholine-induced bronchoconstriction. Inhalation of acetylcholine caused dose-dependent bronchoconstriction in all groups. Pretreatment with phentolamine and/or propranolol had no effect on acetylcholine-induced bronchoconstriction. Values represent the mean ± S.E. for five animals.

3.3. Effect of atropine on the adrenoceptor antagonist-mediated modulations of bronchoconstriction induced by cigarette smoke

The time-course of changes in R_L induced by exposure to cigarette smoke are illustrated in Fig. 2. Modulations of cigarette smoke-induced bronchoconstriction by phentolamine and/or propranolol, as shown in Fig. 1, were not observed in the animals pretreated with atropine.

3.4. Effect of adrenoceptor antagonists on bronchoconstriction induced by exogenously applied acetylcholine

Inhalation of acetylcholine caused dose-dependent bronchoconstriction in all groups (Fig. 3). Pretreatment with phentolamine and/or propranolol had no effect on acetylcholine-induced bronchoconstriction.

4. Discussion

The present study demonstrated that cigarette smoke-induced bronchoconstriction was modulated by pretreatment with adrenoceptor antagonists in guinea pigs. Phentolamine significantly inhibited bronchoconstriction, while propranolol enhanced it. However, phentolamine enhanced

bronchoconstriction in the propranolol-treated animals. Atropine pretreatment completely abolished all modulations of bronchoconstriction by the adrenoceptor antagonists in this study. Furthermore, pretreatment with phentolamine and/or propranolol had no effect on the bronchoconstriction induced by inhaled acetylcholine. Thus, we postulate that this adrenergic modulation acts through pre-junctional inhibition of cholinergic bronchoconstriction.

It has been reported that several components of cigarette smoke inactivate monoamine oxidase, a key enzyme in the metabolism of noradrenaline (Yu and Boulton, 1987). Therefore, it is possible that adrenergic effects are enhanced in cigarette smoke-induced airway responses.

Cigarette smoke-induced bronchoconstriction is mediated by multiple mediators, including tachykinins, acetylcholine, and thromboxane in guinea pigs (Hong et al., 1995; Lee et al., 1995; Hong and Lee, 1996; Matsumoto et al., 1996a,b,c). We previously demonstrated that cigarette smoke-induced bronchoconstriction was completely abolished by tachykinin-receptor antagonist, while atropine partly inhibited bronchoconstriction, suggesting that bronchoconstriction includes a cholinergic component and a noncholinergic component possibly mediated by tachykinins (Matsumoto et al., 1996b).

Although previous studies have reported that the release of tachykinins from airway C-fibers may be inhibited presynaptically via α_2- and β_2-adrenoceptors in guinea pigs (Grundstrom et al., 1984; Matran et al., 1989; Kamikawa and Shimo, 1991; Verleden et al., 1993), the adrenergic nervous system seemed to have no direct inhibitory effect on the release of tachykinins in the present condition. Thus, both phentolamine and propranolol had no effect on the noncholinergic bronchoconstriction which was observed in atropine-treated animals. Indeed, previous investigators have confirmed that phentolamine and propranolol, used in the same doses as in our study, showed no effect on tachykinin-mediated extravasation of plasma into the airways induced by cigarette smoke in guinea pigs (Lei et al., 1995).

The precise mechanism of cholinergic bronchoconstriction is an important issue, because it has not been established whether the cholinergic bronchoconstriction is due to a central reflex or a local effect. Previous studies have shown that bilateral cervical vagotomy and pretreatment with hexamethonium, a ganglionic blocker, partially blocked the cigarette smoke-induced bronchoconstriction (Lee et al., 1995; Hong and Lee, 1996). It is likely that central reflex mechanism is, at least partially, involved in cholinergic bronchoconstriction induced by cigarette smoke. However, the role of local reflex mechanism remains conceivable. We previously showed that atropine was still effective to suppress the histamine-induced bronchoconstriction in vagotomized- or hexamethonium-treated guinea pigs (Inoue et al., 1991). This may suggest the contribution of local reflex in cholinergic bronchoconstriction in guinea pigs. To determine whether local reflex is also important in cigarette smoke-induced bronchoconstriction, further studies are needed.

Previous in vitro studies demonstrated that the cholinergic contraction of airway smooth muscles can be inhibited pre-junctionally by α_2-adrenoceptors in guinea pigs (Grundstrom et al., 1981; Matran et al., 1989; Thompson et al., 1990). Wessler and coworkers have suggested that the cholinergic contraction may be inhibited by inhibitory prostaglandins which are generated from the airway epithelium following stimulation of β_2-adrenoceptors on the epithelium (Wessler et al., 1994). Pre-junctional regulation by multiple adrenoceptor-mediated pathways may have an important role in the regulation of bronchoconstrictor responses. Our data supported this assumption, since propranolol significantly enhanced cigarette smoke-induced bronchoconstriction, and phentolamine, combined with propranolol, allowed for further enhancement of bronchoconstriction. Further investigation is necessary to determine the precise receptor subtypes.

Based on the fact that cholinergic bronchoconstriction can be inhibited by pre-junctional α_2-adrenoceptors on cholinergic nerves, phentolamine pretreatment should enhance cigarette smoke-induced bronchoconstriction. In the present study, however, phentolamine inhibited bronchoconstriction. This discrepancy may be explained by the autoinhibition of noradrenaline release from adrenergic nerves by the α_2-autoreceptor (Hoffman and Lefkowitz, 1980; Starke et al., 1989). In the α_2-blockade state, the release of noradrenaline into the airway would be markedly increased, which may enhance β_2-adrenoceptor-mediated inhibition of acetylcholine release. In contrast, phentolamine enhanced the bronchoconstriction in the animals treated with propranolol. Although phentolamine would partly enhance acetylcholine release by blockade of α_2-heteroceptor-mediated inhibition on the cholinergic nerve, this effect may be overcome by β_2-mediated inhibition. Thus, these complex mechanisms may result in the inhibition of cigarette smoke-induced bronchoconstriction in phentolamine-treated animals, and provide in vivo evidence for a modulatory role of α_2-adrenergic autoregulation in bronchoconstrictor responses. To address this issue, we performed further investigation using α_2-adrenoceptor selective antagonist, yohimbine. Pretreatment with yohimbine showed modulatory effects similar to those of phentolamine, which strongly suggests the important role of α_2-adrenergic autoregulation in cigarette smoke-induced bronchoconstriction.

The dose of cigarette smoke in this study is thought to be higher than that of exposure estimated in humans. Bronchoconstriction induced by conventional smoking was reported to be significant but very weak (Nadel and Comroe, 1961). The results of this study might not be directly interpolated to humans. However, this study demonstrated the protective role of adrenergic system against cigarette smoke-induced bronchoconstriction. Exposure to cigarette smoke may cause considerable bronchoconstriction in some

patients treated with adrenergic blockers for hypertension, arrhythmia and hyperthyroidism. Therefore, this study may have potential significance to elucidate the adverse effects of cigarette smoking in humans.

The modulatory effects of adrenergic mechanism may be mediated via airway sympathetic nerves or via circulating catecholamine (Piper et al., 1967; Drazen, 1978). It is well known that cigarette smoking rapidly releases adrenaline from the adrenal medulla in humans (Cryer et al., 1976). Although the contribution of circulating catecholamine remains uncertain in guinea pigs, the modulatory effect of α_2-adrenergic autoregulation shown in the present study suggests that sympathetic nervous system may play a key role in cigarette smoke-induced bronchoconstriction.

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