

Role of GABA receptors in the bronchial response: studies in sensitized guinea-pigs

Y. TOHDA, K. OHKAWA, H. KUBO, M. MURAKI, M. FUKUOKA and S. NAKAJIMA

Fourth Department of Internal Medicine, Kinki University School of Medicine, Osaka, Japan

Summary

Background γ -Aminobutyric acid (GABA), an important inhibitory neurotransmitter in the mammalian central nervous system, is also found in peripheral tissues, including the lung. GABA has recently been shown to modulate the contraction of airway smooth muscle. **Objective** We studied the effect of GABA on the contractile properties of tracheal smooth muscle by measuring the tension of the trachea isolated from non-sensitized and ovalbumin (OA)-sensitized guinea-pigs under isometric conditions.

Methods Guinea-pigs were sensitized by intraperitoneal doses of OA to prepare a bronchial asthma model. Tracheal spiral rings were prepared from the OA-sensitized as well as normal, non-sensitized guinea-pigs. Using the tracheal preparations, the effects of GABA and GABA_A and GABA_B receptor agonists (muscimol and baclofen) and antagonists (bicuculline and saclofen) on the basal tone of the trachea and on tracheal contraction induced by electrical field stimulation (EFS) were determined. The effect of GABA on tracheal contraction induced by exogenous acetylcholine was also studied.

Results GABA and GABA agonists and antagonists had no effect on the basal tone of normal guinea-pig tracheae. Both GABA_A and GABA_B receptor agonists, as well as GABA, suppressed EFS-induced contraction of normal guinea-pig tracheae in a reversible, dose-dependent manner. Moreover, this suppression was reserved to the control level by either GABA_A and GABA_B receptor antagonists. In tracheal spiral ring prepared from OA-sensitized guinea-pigs, GABA and baclofen caused a smaller reversible inhibition of EFS-induced contraction than in normal tracheal spiral ring, while muscimol inhibited EFS-induced tracheal contraction to a similar extent to that observed in normal tracheae. GABA had no effect on the tracheal contractile response to acetylcholine.

Conclusion The results suggest that there may be a biological mechanism mediated by prejunctional GABA_B receptors which attenuates cholinergic contraction of airway smooth muscle and that dysfunction of the receptors may underlie the airway obstruction in asthmatics.

Keywords: bronchial asthma, autonomic disturbance, GABA receptor, GABA_B receptor

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Introduction

γ -Aminobutyric acid (GABA) is an inhibitory neurotransmitter in the mammalian central nervous system. GABA causes postsynaptic suppression of nervous activity by

inducing hyperpolarization in the superior part of the central nervous system, while it reduces neurotransmitter release through depolarization of primary afferent nerve terminals in the spinal cord. The common mechanism of these effects is activation of chloride channels and a consequent increase in chloride permeation. Therefore, impairment of GABA-mediated neurotransmission may lead to the development of CNS disorders, including convulsions. Bowery and Brown first found the presence of GABA receptors in the peripheral

Correspondence: Dr Y. Tohda, Fourth Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohnohigashi, Osakasayama, Osaka 589, Japan.

nervous system by recording GABA-induced transient depolarization of rat superior cervical ganglia, which were selectively blocked by bicuculline [1]. Particularly high GABA levels as observed in the CNS are observed in the Auerbach's plexus, epithelial cells of salpinx, and β cells of pancreas [2].

GABA receptors have two subtypes, GABA_A receptors sensitive to bicuculline and GABA_B receptors insensitive to bicuculline. Via GABA_A receptors, GABA increases chloride permeation and thereby suppresses excessive cell excitement. Actions mediated by GABA_B receptors are independent of chloride ions and include suppression of calcium channels coupled with G protein. Bowery *et al.* reported the presence of GABA_B receptors in the peripheral nervous system [3].

It has been reported that GABA modulates the tone of airway smooth muscle [4,5]. In the present study, we determined the effects of GABA and related compounds on electrically induced contraction of tracheal spiral ring prepared from ovalbumin (OA)-sensitized asthmatic guinea-pigs to examine the roles of GABA receptors in the aetiology of bronchial asthma.

Materials and methods

Animals

Forty-five healthy male Hartley guinea-pigs were purchased from SRL Japan Inc., Hamamatsu, Japan. Twenty of them were sensitized to OA according to the procedures previously described by Tohda [6]. Guinea-pigs received intraperitoneal injections of 30 mg/kg cyclophosphamide (Shionogi Pharmaceutical Co. Ltd, Osaka, Japan). Two days later, 1 mg/0.5 mL OA (grade V; Sigma Chemical Co. Ltd, St Louis, MO, USA) containing 100 mg aluminum hydroxide as an adjuvant (Wako Pure Chemical Industries, Osaka, Japan) was injected intraperitoneally. Three weeks later, 10 μ g/0.5 mL OA containing 100 mg aluminum hydroxide was injected intraperitoneally for sensitization. The OA-sensitized animals were used in the study at 3 weeks after the last sensitizing dose was given. The 45 animals, including 25 non-sensitized ones, weighed 400–500 g when they were sacrificed for use in the study.

Test compounds

The following compounds were purchased from Wako Pure Chemicals Co., Ltd, Osaka, Japan for use in the study: indomethacin, muscimol, baclofen, bicuculline, and saclofen.

Preparation of guinea-pig tracheal spiral ring

The animals were stunned by a blow on the head and exsanguinated to death by severing the carotid artery. The

trachea was immediately removed from each animal and cut into a ring 5 mm in breadth. Each spiral ring was suspended with a tension of 1 g using tungsten wire in an organ bath containing 20 mL of modified Krebs solution (pH 7.4; NaCl 118 mM, KCl 4.6 mM, MgSO₄ 7H₂O 1.1 mM, CaCl₂ 2H₂O 1.8 mM, NaHCO₃ 24.9 mM, KH₂PO₄ 1 mM, glucose 11.1 mM) which was maintained at 37°C and aerated with mixed O₂/CO₂ (95:5) gas. To the modified Krebs solution was added indomethacin (2×10^{-6} M) to block the effects of endogenous prostaglandins. In the bath, the spiral ring was positioned between two platinum electrodes to deliver electrical field stimulation (EFS).

Measurement of tension of tracheal spiral rings

Isometric tension measurements of the tracheal smooth muscle were made using an isometric transducer (TB-651T, Nihon Koden, Tokyo) connected to the end of the tracheal spiral ring and were recorded on a thermal recorder (RTA-1200M, Nihon Koden, Tokyo) via a specific amplifier (EF-60G, Nihon Koden, Tokyo). Prior to the measurement, each preparation was stabilized in the bath for 60 min.

Effects of GABA and GABA receptor agonists (muscimol and baclofen) and antagonists (bicuculline and saclofen) on the basal tone of tracheal spiral rings

To assess the effects of GABA and GABA receptor agonists and antagonists on the basal tone of tracheal spiral smooth muscle, the tracheal tension was measured in tracheal spiral ring which were prepared from non-sensitized guinea-pigs and exposed to increasing concentrations of these compounds (1×10^{-10} – 10^{-3} M).

Effect of GABA on tracheal contraction induced by exogenous acetylcholine

The effect of pretreatment with GABA (10^{-4} M) on the tracheal contraction induced by acetylcholine (10^{-9} – 10^{-3} M) was assessed to determine whether GABA acts at the prejunctional or postjunctional level of cholinergic tracheal contraction.

All contractile responses are expressed as the percentages relative to the response to 10^{-3} M of acetylcholine without prior treatment with GABA (maximum baseline response; control).

Effects of GABA and GABA receptor agonists and antagonists on EFS-induced tracheal contraction

EFS

Tracheal contraction was induced by electrical field stimulation (EFS) of the nerves present in the isolated

tracheal smooth muscle. From an electrical stimulator (SEN-7203, Nihon Koden, Tokyo, Japan), rectangular pulses (pulse width 0.5 ms, 50 V) of varying frequencies were delivered to the field.

Effects of GABA and GABA receptor agonists

The effects of increasing concentrations (1×10^{-10} – 10^{-3} M) of GABA, muscimol (a GABA_A receptor agonist) and baclofen (a GABA_B receptor agonist) on EFS-induced tracheal contraction were determined using tracheal spiral ring prepared from non-sensitized and OA-sensitized guinea-pigs. For each test compound, the concentration in the organ bath was increased to the next higher level at 10 min intervals. At 5 min after each concentration was achieved, rectangular pulses (5 Hz) were delivered for 15 s. All contractile responses are expressed as the percentages relative to the EFS-induced response following treatment with distilled water (baseline response; control).

Effects of GABA receptor antagonists

The effects of bicuculline (a GABA_A receptor antagonist) and saclofen (a GABA_B receptor antagonist) on the tracheal response to GABA were determined using tracheal spiral ring prepared from non-sensitized guinea-pigs.

Baseline tracheal responses to EFS given for 15 s were recorded while the pulse frequency was increased gradually from 3 to 20 Hz at 10 min intervals. The IC_{50} of GABA for each frequency of stimulation was determined from the baseline data and was used in the experiment. At 10 min after application of GABA, bicuculline (10^{-5} M) or saclofen (10^{-5} M) was added to the organ bath. After 20 min of incubation, the tracheal response to EFS given at each frequency was recorded and compared with the baseline response. All contractile responses are expressed as the percentages relative to the baseline response to 20 Hz of EFS (maximum baseline response; control).

Statistical analysis

All data are expressed as the means of five preparations $\pm$ standard deviation. Statistical analysis was performed using Student's *t*-test (two-tailed). Data were considered significant when the *P*-value was not more than 0.01.

Results

Effects of GABA and GABA receptor agonists and antagonists on the basal tone of tracheal spiral ring

GABA, muscimol, baclofen, bicuculline and saclofen had no effects on the basal tone of tracheal spiral ring (data not shown).

Effect of GABA on tracheal contraction induced by exogenous acetylcholine

The dose–response curves for tracheal contractile response to acetylcholine (10^{-9} – 10^{-3} M) in the presence and absence of GABA are shown in Fig. 1. GABA did not alter the dose–response curve for acetylcholine-induced tracheal contraction.

Effects of GABA and GABA receptor agonists and antagonists on EFS-induced tracheal contraction

Studies using tracheal spiral rings prepared from non-sensitized animals

Effects of GABA and GABA receptor agonists on EFS-induced tracheal contraction (Fig. 2)

GABA suppressed tracheal contractile responses to EFS in a dose-dependent manner. The suppressions caused by 10^{-9} M or higher concentrations of GABA were statistically significant compared with the control ($P < 0.01$). Baclofen, a GABA_B receptor agonist, also suppressed tracheal contractile responses to EFS significantly at concentrations of 10^{-9} M or more ($P < 0.01$). Muscimol, a GABA_A receptor agonist, caused significant suppressions at concentrations of 10^{-5} M ($P < 0.01$).

Effects of GABA receptor antagonists on tracheal responses to GABA

Bicuculline, a GABA_A receptor antagonist, completely abolished the suppression of EFS-induced tracheal contraction by GABA (Fig. 3). Saclofen, a GABA_B receptor antagonist, also antagonized the suppressive effect of GABA on EFS-induced tracheal contraction (Fig. 4).

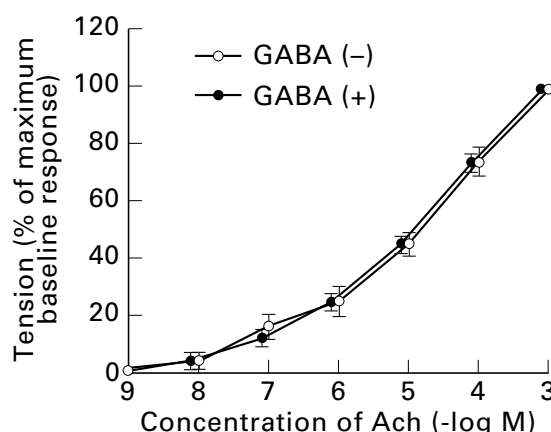


Fig. 1. Effect of GABA on tracheal contraction induced by exogenous acetylcholine. GABA had no significant effect on acetylcholine-induced tracheal contraction as assessed in tracheal spiral ring prepared from non-sensitized guinea-pigs.

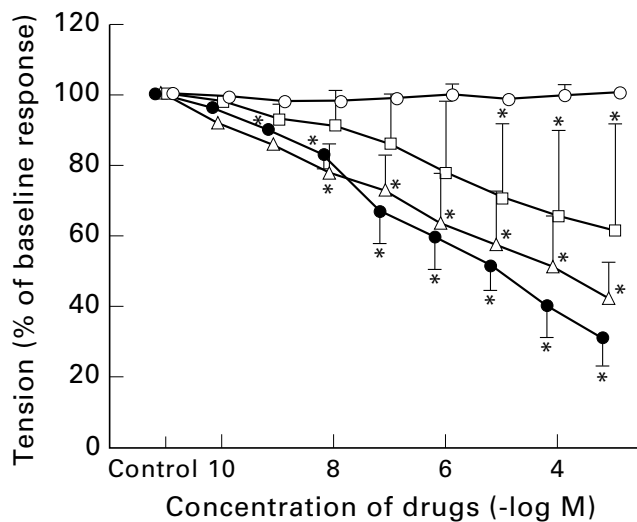


Fig. 2. Effect of GABA and GABA receptor agonists on EFS-induced tracheal contraction. GABA and GABA receptor agonists suppressed EFS-induced tracheal contraction in a dose-dependent manner in tracheal spiral ring prepared from non-sensitized guinea-pigs. * $P < 0.01$ vs control.

Studies using tracheal spiral rings prepared from OA-sensitized guinea-pigs

Effects of GABA on EFS-induced tracheal contraction
GABA caused a significant and dose-dependent suppression of tracheal contractile responses to EFS ($P < 0.01$). However, the suppression was significantly smaller than that observed in the tracheal spiral ring prepared from non-sensitized animals ($P < 0.01$; Fig. 5).

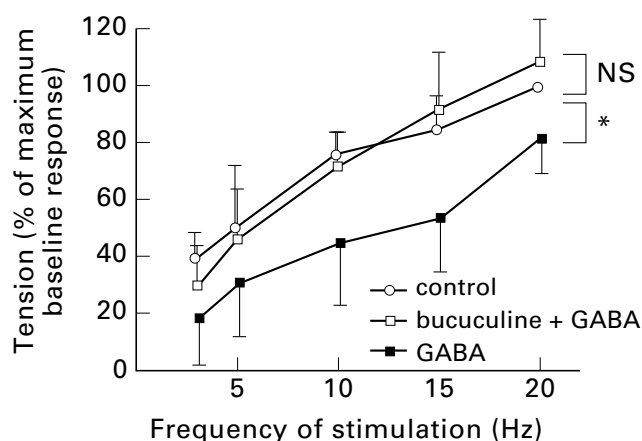


Fig. 3. Effect of bicuculline, a GABA_A receptor antagonist, on the suppression of EFS-induced tracheal contraction by GABA. Bicuculline completely abolished the suppression of EFS-induced tracheal contraction by GABA in tracheal spiral ring prepared from non-sensitized guinea-pigs. The statistical analysis was by area-under-curve (AUC). * $P < 0.01$. NS. No significant difference.

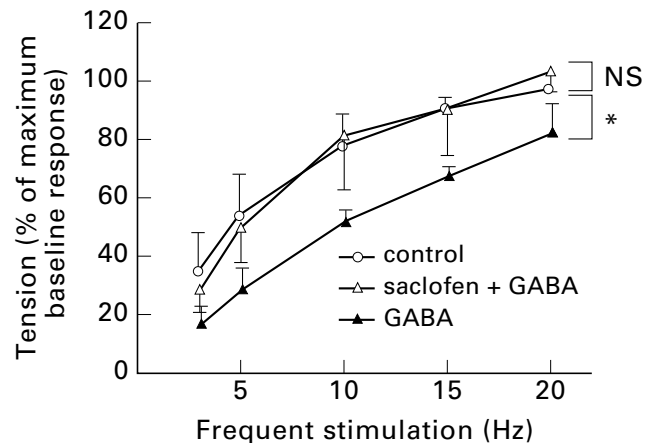


Fig. 4. Effect of saclofen, a GABA_B receptor antagonist, on the suppression of EFS-induced tracheal contraction by GABA. Saclofen completely abolished the suppression of EFS-induced tracheal contraction by GABA in tracheal spiral ring prepared from non-sensitized guinea-pigs. The statistical analysis was by area-under-curve (AUC). * $P < 0.01$. NS, No significant difference.

Effects of GABA receptor agonists on EFS-induced tracheal contraction

The GABA_A receptor agonist muscimol significantly suppressed EFS-induced tracheal contraction ($P < 0.01$). The suppression caused by muscimol did not differ significantly from that observed in the tracheal spiral ring prepared from non-sensitized animals (Fig. 6). On the other hand, the

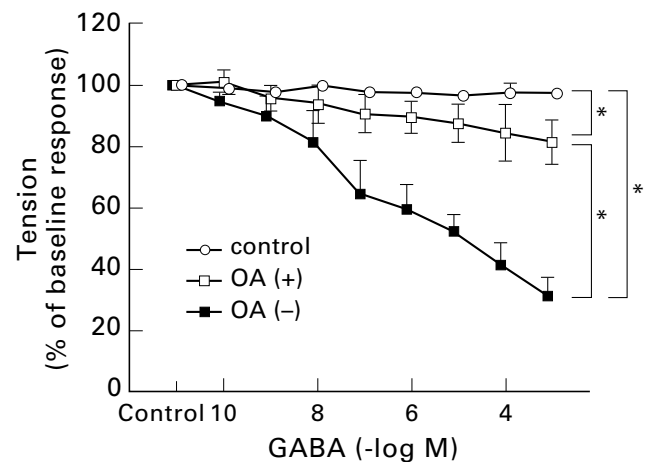


Fig. 5. Effect of GABA on EFS-induced tracheal contraction assessed in tracheal spiral ring prepared from OA-sensitized guinea-pigs. GABA significantly suppressed EFS-induced tracheal contraction in tracheal spiral ring prepared from OA-sensitized guinea pigs but the suppression was significantly smaller than that observed in preparations from non-sensitized guinea-pigs. The statistical analysis was by area-under-curve (AUC). * $P < 0.01$.

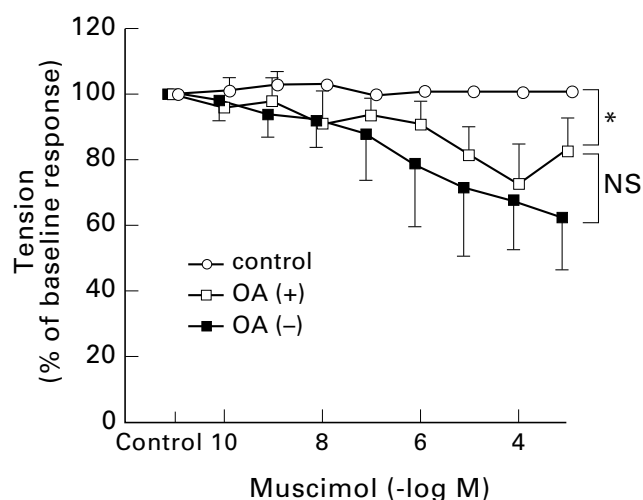


Fig. 6. Effect of muscimol on EFS-induced tracheal contraction assessed in tracheal spiral ring prepared from OA-sensitized guinea-pigs. Muscimol significantly suppressed EFS-induced tracheal contraction in tracheal spiral ring prepared from OA-sensitized guinea-pigs and the suppression did not differ significantly from that observed in tracheal preparations from non-sensitized guinea-pigs. The statistical analysis was by area-under-curve (AUC). * $P < 0.01$. NS. No significant difference.

GABA_B receptor agonist baclofen caused a significantly smaller suppression of EFS-induced tracheal contraction than that caused in the tracheal spiral ring prepared from non-sensitized animals ($P < 0.01$; Fig. 7). There were no significant differences between non-sensitized and

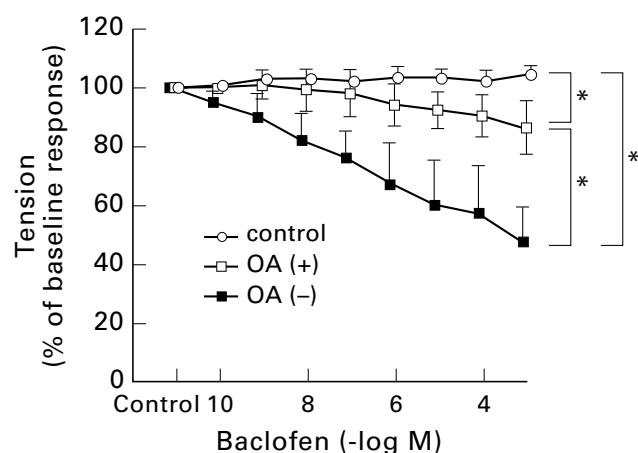


Fig. 7. Effect of baclofen on EFS-induced tracheal contraction assessed in tracheal spiral ring prepared from OA-sensitized guinea-pigs. Baclofen significantly suppressed EFS-induced tracheal contraction in tracheal spiral ring prepared from OA-sensitized guinea-pigs but the suppression was significantly smaller than that observed in tracheal preparations from non-sensitized guinea-pigs. The statistical analysis was by area-under-curve (AUC). * $P < 0.01$.

sensitized trachealis muscle as to cholinergic and EFS contractile response.

Discussion

In recent years, airway inflammation has been recognized as the primary pathological abnormality underlying bronchial asthma [7]. Nevertheless, it is still true that airway obstruction is caused by contraction of bronchial smooth muscle which may be mediated by the autonomic nervous system. Failure of autonomic control of airway smooth muscle tone has been reported to result from cholinergic stimulation [8,9], α -adrenergic stimulation [10], suppression of β -adrenergic activity [11], and dysfunction of muscarinic M2-receptors [12]. Airway inflammation may enhance autonomic disturbance in the modulation of airway smooth muscle tone since inflammatory mediators have been reported to upregulate muscarinic acetylcholine receptors [13,14] and histamine receptors [14]. The present study focused on the role of GABA, a known autonomic neurotransmitter or nervous activity modifying substance, in the modulation of tracheal smooth muscle tone and the function of GABA receptors in a bronchial asthma model. All experiments were performed *in vitro* using tracheal spiral rings suspended organ baths containing modified Krebs's solution, which contained indomethacin to prevent indirect effect of prostaglandins.

In the present study, GABA and the two GABA receptor agonists, muscimol and baclofen, had no significant effects on basal tone of normal tracheae. This was in agreement with the findings reported by Tamaoki *et al.* [4] and Chapman *et al.* [15]. On the other hand, Giotti *et al.* [16] reported that guinea-pig intestinal smooth muscle was contracted by GABA and muscimol and relaxed by baclofen. This apparent discrepancy may be accounted for by different roles of GABA in the trachea and the intestine. GABA released from the nerves in the Auerbach's plexus may not act as an inhibitory neurotransmitter but may act to promote intestinal motility [2].

In the study of the level of action of GABA, GABA did not antagonize acetylcholine-induced tracheal contraction, indicating that the suppressive effect of GABA on tracheal contraction is exerted primarily at the prejunctional level. This finding agrees with that reported by Tamaoki *et al.* [4] but may contradict the suggestion of a postjunctional action of GABA by Peter *et al.* [17], who observed that baclofen reduced bronchial contractile response to methacholine in patients with injured cervical spinal cord.

Although GABA and the two GABA agonists, muscimol and baclofen significantly suppressed EFS-induced tracheal contraction, the GABA_B receptor agonist baclofen seemed to be more potent than the GABA_A receptor agonist muscimol in suppressing tracheal contraction because significant suppressions were caused by lower concentrations of baclofen compared to muscimol. Chapman *et al.* [15] reported that a

GABA_A receptor agonist had no effect on EFS-induced tracheal contraction, while Tamaoki *et al.* [4] reported that muscimol also reduced the tracheal response to EFS, which is consistent with the finding obtained in the present study.

The GABA_A and GABA_B receptor antagonists, bicuculline and saclofen, completely blocked the suppressive effect of GABA on EFS-induced tracheal contraction. Ong *et al.* [18] found a bicuculline-sensitive, Cl⁻-dependent effect of baclofen (a typical GABA_B receptor agonist) mediated by GABA_A receptors in the trachea and pointed out that GABA agonists have non-selective affinity for the two receptor subtypes in the airway.

Since GABA in itself did not cause tracheal contraction, the GABA-mediated modulation of airway smooth muscle seems to occur by a mechanism different from such a dual control via GABA_A (promotion) and GABA_B receptors (suppression) that is observed over intestinal and vesical motility. Bicuculline and saclofen, respectively, are thought to be highly selective in blocking GABA_A and GABA_B receptor-mediated effects [19]. Therefore, the blockade of the suppressive effect of GABA on tracheal contraction by both antagonists observed in the present study suggests that the roles of the two GABA receptor subtypes in the airway are different from those in other organs.

The suppression of EFS-induced tracheal contraction by GABA in the preparations from OA-sensitized animals was significantly smaller than that in the preparations from non-sensitized animals. This suggests dysfunction of GABA receptors in the airways of OA-sensitized animals, which may, at least in part, contribute to the autonomic disturbance in bronchial asthma. In addition, the suppression of EFS-induced tracheal contraction by baclofen was significantly smaller in the system prepared from OA-sensitized animals, while the suppressions caused by muscimol were similar in tracheal preparations from OA-sensitized and non-sensitized animals. Therefore, the dysfunction of GABA receptors in the airway in OA-sensitized animals may primarily be due to dysfunction of GABA_B receptors.

Although further studies, such as measurement of receptor subtype expression, are needed (or are under way), the present results suggest that dysfunction of GABA-receptors, mainly GABA_B receptor in the airways may contribute to the airway obstruction in asthmatics.

Acknowledgments

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References

- 1 Bowery NG, Brown DA. Depolarizing action of γ -aminobutyric acid and related compounds on rat superior cervical ganglia in vitro. *Br J Pharmacol* 1974; 50:205–18.
- 2 Tanaka C, Nishizuka Y. Signal transduction in organisms. Tokyo: Nankodo, 1993.
- 3 Bowery NG, Doble A, Hill DR *et al.* Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *Eur J Pharmacol* 1981; 71:53–70.
- 4 Tamaoki J, Graf PD, Nadel JA. Effect of γ -aminobutyric acid on neurally mediated contraction of guinea pig trachealis smooth muscle. *J Pharmacol Exp Ther* 1987; 243:86–90.
- 5 Belvisi MG, Ichinose M, Barnes PJ. Modulation of non-adrenergic, non-cholinergic neural bronchoconstriction in guinea-pig airways via GABA_B-receptors. *Br J Pharmacol* 1989; 97:1225–31.
- 6 Tohda Y. Experimental study of the etiology of bronchial asthma: study using a model of biphasic asthmatic response. *Med J Kinki Univ* 1989; 14:133–49.
- 7 Makino S. Guidelines for the treatment of allergic disease. Tokyo: Life Science Medica, 1993.
- 8 Kaliner M. The cholinergic nervous system and immediate hypersensitivity. I. Eccrine sweat responses in allergic patients. *J Allergy Clin Immunol* 1975; 58:308–15.
- 9 Smith LJ, Schelhamer JH, Kaliner M. Cholinergic nervous system and immediate hypersensitivity. II. Analysis of pupillary responses. *J Allergy Clin Immunol* 1980; 66:374–8.
- 10 Henderson WR, Shelhamer JH, Reingold DB *et al.* Alpha-adrenergic hyperresponsiveness in asthma. Analysis of vascular and pupillary responses. *New Engl J Med* 1980; 302:1390–6.
- 11 Szentivanyi A. The beta adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy* 1968; 42:203–32.
- 12 Minette PA, Barnes PJ. Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways. *J Appl Physiol* 1988; 64:2532–7.
- 13 Takehana Y, Kikuchi S, Kusama H *et al.* Effect of (E)-3-[p-(1H-imidazol-1-ylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate, a specific thromboxane (TX) A₂ synthase inhibitor, on antigen-induced bronchial hyperresponsiveness in guinea-pigs. *Clin Report* 1990; 24:461–9.
- 14 Sugihara R. Role of muscarinic acetylcholine receptor subtypes in the etiology of bronchial asthma. *Med J Kinki Univ* (in press).
- 15 Chapman RW, Hey JA, Rizzo CA, Bolser DC. GABA_B receptors in the lung. *TIPS* 1993; 14:26–9.
- 16 Giotti A, Luzzi S, Zilletti L. GABA_A and GABA_B receptor-mediated effects in guinea-pig ileum. *Br J Pharmacol* 1983; 78:469–78.
- 17 Peter VD, Ann MS, William AB *et al.* Inhibition of bronchial hyperresponsiveness by the GABA-agonist baclofen. *Chest* 1994; 106:758–61.
- 18 Ong J, Kerr DIB. GABA receptors in peripheral tissue. *Life Sci* 1990; 46:1489–501.
- 19 Akaike N. Control of GABA receptors by intracellular factors. *Folia Pharmacol Jpn* 1992; 99:275–85.