Y-27632 augments the isoflurane-induced relaxation of bronchial smooth muscle in rats

Motohiko HANAZAKI1, Yoshihiko CHIBA2, Masataka YOKOYAMA1, Kiyoshi MORITA1, Atsushi KOHJITANI3, Hiroyasu SAKAI2 and Miwa MISAWA2

1Department of Anesthesiology and Resuscitology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
2Department of Pharmacology, School of Pharmacy, Hoshi University
3Department of Dental Anesthesiology, Kagoshima University Graduate School of Medical and Dental Sciences

Received September 8, 2008; Accepted October 27, 2008

Abstract

Recent studies revealed an involvement of RhoA/Rho-kinase signaling in the agonist-induced Ca\textsuperscript{2+} sensitization of bronchial smooth muscle contraction, and the pathway has now been proposed as a new target for the treatment of airway obstructive diseases, such as asthma. On the other hand, volatile anesthetics such as isoflurane are traditionally used to treat status asthmaticus. In the present study, the effect of inhibition of Rho-kinase on the isoflurane-induced relaxation of bronchial smooth muscle was investigated. Smooth muscle strips of intrapulmonary bronchi obtained from Wistar rats were used. Application of isoflurane (0.5–4.0\%, generated by a calibrated vaporizer) to the acetylcholine (30 \(\mu\text{M}\))-precontracted rat bronchial smooth muscles caused a concentration-dependent relaxation. Interestingly, the isoflurane-induced relaxation was significantly augmented by the pretreatment with subthreshold concentration of Y-27632, a Rho-kinase inhibitor. Thus, the combined use of Y-27632 and isoflurane might be useful for treatment of severe airway blockade, such as status asthmaticus.

Key words: Y-27632, isoflurane, acetylcholine, bronchial smooth muscle, rat

Introduction

Increased airway narrowing in response to nonspecific stimuli, called airway hyperresponsiveness, is a characteristic feature of allergic bronchial asthma. One of the factors that contribute to the exaggerated airway narrowing in asthmatics is an abnormality of the nature of airway smooth muscle (Martin et al., 2000; Seow et al., 1998). Despite the recent asthma management by pharmacological agents such as \(\beta\)-stimulants and corticosteroids, status asthmaticus continues to be a life-threatening event. In addition, anesthesiologists frequently

Correspondence to: Motohiko Hanazaki, M.D., Department of Anesthesiology and Resuscitology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikatacho, Okayama 700-8558, Japan
Phone: +81-86-235-7778 Fax: +81-86-235-6984 e-mail: motohiko@hanazaki.com
experience severe bronchospasm when an endotracheal intubation was conducted for mechanical ventilation in asthmatic patients.

Volatile anesthetics such as isoflurane are traditionally used to treat status asthmaticus (Maltais et al., 1994) and their use is recommended when general anesthesia is required for patients with asthma. Although the mechanism(s) of the bronchodilatation induced by volatile anesthetics is not fully understood, they could inhibit both Ca²⁺-dependent and Ca²⁺-independent contraction in airway smooth muscles (Akao et al., 1996; Hanazaki et al., 2000; Kai et al., 1998). To date, there is increasing evidence that a monomeric GTP-binding protein RhoA and its downstream target Rho-kinase are involved in the Ca²⁺-independent contraction, i.e., agonist-induced Ca²⁺ sensitization, in airway smooth muscle (Chiba et al., 1999; 2005, Ito et al., 2001; Yoshii et al., 1999). Recently, we found that Rho-kinase inhibitors augmented the relaxation induced by propofol, an intravenous anesthetic agent that is also able to relax airway smooth muscle, in rat bronchial smooth muscle (Hanazaki et al., 2008). The findings indicate that administration of subanesthetic dose of anesthetic agent and a Rho-kinase inhibitor together may rescue asthmatics from the airway blockade. On the other hand, volatile anesthetics would have advantage because they act on airways at first. So in the present study, to determine whether the inhibition of Rho-kinase also augments the relaxation of bronchial smooth muscle induced by volatile anesthetics, effect of a Rho-kinase inhibitor Y-27632 (Uehata et al., 1997) on the isoflurane-induced relaxation was investigated.

**Methods**

Male Wistar rats (6 weeks of age, 180–220 g, specific pathogen-free) were used. All experiments were approved by the Institutional Animal Care and Use Committee at Okayama University (Okayama, Japan). Animals were killed by exsanguination from the abdominal aorta under anesthetization by sodium pentobarbital (50 mg/kg, i.p.). The third branch of the intrapulmonary bronchus was isolated by the method as previously described (Chiba et al., 1999). In brief, the tissue was carefully cleaned of lung parenchyma and adhering connective tissue, and then cut into ring strips (about 200 µm width, 500 µm diameter). The epithelium was removed by gently rubbing with keen-edged tweezers under a stereomicroscope. The resultant tissue ring preparation was suspended in a 400-µl organ bath at a resting tension of 50 mg. The isometric contraction of the circular smooth muscle was measured with a force-displacement transducer (T7-8-240, Orientec, Japan) and recorder (DC-3100, NEC-sanei, Japan). The organ bath contained modified Krebs–Henseleit solution with the following composition (mM); NaCl 118.0, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2 and glucose 11.0 (pH 7.4). The buffer solution was oxygenated with 95% O₂–5% CO₂ at room temperature. During an equilibration period in the organ bath, the tissues were washed four times at 15 min intervals and were equilibrated slowly to a baseline tension of 50 mg. After the equilibration period, 30 µM acetylcholine (ACh), which produce approximately 50% of the maximal ACh-induced contraction (data not shown), was applied to the tissue. Isoflurane (0.5–4.0%; Abott Japan, Osaka, Japan), vaporized by 95% O₂–5% CO₂ via a calibrated vaporizer, was cumulatively applied when a stable contraction with 30 µM ACh was obtained. Y-27632 (1 µM; Calbiochem, La Jolla,
CA) or its vehicle was added to isoflurane.

Relaxation was expressed as a percent change from the initial force produced by 30 µM ACh. Data are expressed as the mean ± S.D. Statistical significance of difference between groups was determined by two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test with *SigmaStat* 3.0 for Windows (SPSS Inc., Chicago, IL, United States) and a value of *P*<0.05 was considered as significant.

**Results and discussion**

Recently, an involvement of RhoA/Rho-kinase signaling in the agonist-induced Ca²⁺ sensitization of bronchial smooth muscle contraction has been suggested. Our previous studies revealed an augmentation of the RhoA/Rho-kinase-mediated Ca²⁺ sensitization in animal models of allergic bronchial asthma (Chiba et al., 1999; 2005), and the RhoA/Rho-kinase pathway has now been proposed as a new target for the treatment of airway hyperresponsiveness in asthma (Gosens et al., 2006). On the other hand, volatile anesthetics are traditionally used to treat airway blockade, such as status asthmaticus (Maltais et al., 1994), although the mechanism of the bronchodilatation induced by these agents is not fully understood. Currently, we examined whether the combined use of Rho-kinase inhibitors and volatile anesthetics is beneficial for the treatment of bronchoconstriction.

As previously reported in canine tracheal (Kai et al., 1998; Yamakage et al., 1993) and human bronchial smooth muscles (Mercier et al., 2002), a concentration-dependent relaxation induced by isoflurane was observed in rat bronchial smooth muscle precontracted with ACh (Fig. 1, *closed circles*). Reportedly, volatile anesthetics have an ability to inhibit L-type voltage-
operated Ca\textsuperscript{2+} channels (Yamakage et al., 1993). In addition, it has also been suggested that volatile anesthetics inhibit agonist-induced Ca\textsuperscript{2+} sensitization of contraction in airway smooth muscle (Kai et al., 1998; Hanazaki et al., 2000). On the other hand, our previous study revealed that the L-type voltage-operated Ca\textsuperscript{2+} channels are barely involved in the ACh-induced contraction in rat bronchial smooth muscle (Chiba and Misawa, 1995). Thus, the relaxant effect of isoflurane may mainly be mediated by the inhibition of ACh-induced Ca\textsuperscript{2+} sensitization of bronchial smooth muscle contraction in rats.

In the present study, the effect of pretreatment with Y-27632, a Rho-kinase inhibitor (Uehata et al., 1997), on the isoflurane-induced relaxation was examined in rat bronchial smooth muscles. Y-27632 itself, at the concentration used (1 µM), had no significant effect on the ACh-induced contraction (Chiba et al., 2001; Hanazaki et al., 2008). However, as shown in Fig. 1, the isoflurane-induced relaxation was augmented by the subthreshold concentration of Y-27632: the isoflurane concentration-response curve was significantly shifted to the left in the presence of 1 µM Y-27632 (Fig. 1, open vs. closed circles; P<0.001). Although the mechanism of the augmentation of relaxation is not clear here, the results may suggest some synergistic effects between the Rho-kinase inhibition by Y-27632 (Uehata et al., 1997) and unknown inhibitory effect(s) on Ca\textsuperscript{2+} sensitization by volatile anesthetics (Akao et al., 1996; Hanazaki et al., 2001). Alternatively, isoflurane may also have an ability to inhibit RhoA/Rho-kinase-mediated Ca\textsuperscript{2+} sensitization in bronchial smooth muscle cells. Further studies are needed to make clear the mechanism of action of isoflurane.

In conclusion, the isoflurane-induced relaxation of bronchial smooth muscle was significantly augmented in the presence of subthreshold concentration of Y-27632. The combined use of Rho-kinase inhibitors and isoflurane might thus be useful for treatment of severe airway blockade, such as status asthmaticus.

References

Akao, M., Hirasaki, A., Jones, K.A., Wong, G.Y., Bremerich, D.H. and Warner, D.O. (1996). Halothane reduces myofilament Ca\textsuperscript{2+} sensitivity during muscarinic receptor stimulation of airway smooth muscle. Am. J. Physiol. 271: L719–L725.

Chiba, Y. and Misawa, M. (1995). Alteration in Ca\textsuperscript{2+} availability involved in antigen-induced airway hyperresponsiveness in rats. Eur. J. Pharmacol. 278: 79–82.

Chiba, Y., Takada, Y., Miyamoto, S., Mitsui-Saito, M., Karaki, H. and Misawa, M. (1999). Augmented acetylcholine-induced, Rho-mediated Ca\textsuperscript{2+} sensitization of bronchial smooth muscle contraction in antigen-induced airway hyperresponsive rats. Br. J. Pharmacol. 127: 597–600.

Chiba, Y., Takeyama, H., Sakai, H. and Misawa, M. (2001). Effects of Y-27632 on acetylcholine-induced contraction of intact and permeabilized intrapulmonary bronchial smooth muscles in rats. Eur. J. Pharmacol. 427: 77–82.

Chiba, Y., Ueno, A., Shinozaki, K., Takeyama, H., Nakazawa, S., Sakai, H. and Misawa, M. (2005). Involvement of RhoA-mediated Ca\textsuperscript{2+} sensitization in antigen-induced bronchial smooth muscle hyperresponsiveness in mice. Respir. Res. 6: Art. No. 4.

Gosens, R., Schaufsma, D., Nelemans, S.A. and Halayko, A.J. (2006). Rho-kinase as a drug target for the treatment of airway hyperresponsiveness in asthma. Mini Rev. Med. Chem. 6: 339–348.

Hanazaki, M., Jones, K.A., Perkins, W.J. and Warner, D.O. (2001). Halothane increases smooth muscle
protein phosphatase in airway smooth muscle. *Anesthesiology* **94**: 129–136.

Hanazaki, M., Jones, K.A. and Warner, D.O. (2000). Effects of intravenous anesthetics on Ca\(^{2+}\) sensitivity in canine tracheal smooth muscle. *Anesthesiology* **92**: 133–139.

Hanazaki, M., Yokoyama, M., Morita, K., Kohjitani, A., Sakai, H., Chiba, Y. and Misawa, M. (2008). Rho-kinase (ROCK) inhibitors augment the inhibitory effect of propofol on rat bronchial smooth muscle contraction. *Anesth. Analg.* **106**: 1765–1771.

Ito, S., Kume, H., Honjo, H., Katoh, H., Kodama, I., Yamaki, K. and Hayashi, H. (2001). Possible involvement of Rho kinase in Ca\(^{2+}\) sensitization and mobilization by MCh in tracheal smooth muscle. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **280**: L1218–L1224.

Kai, T., Bremerich, D.H., Jones, K.A. and Warner, D.O. (1998). Drug-specific effects of volatile anesthetics on Ca\(^{2+}\) sensitization in airway smooth muscle. *Anesth. Analg.* **87**: 425–429.

Maltais, F., Sovilj, M., Goldberg, P. and Gottfried, S.B. (1994). Respiratory mechanics in status asthmaticus. Effects of inhalational anesthesia. *Chest* **106**: 1401–1406.

Martin, J.G., Duguet, A. and Eidelman, D.H. (2000). The contribution of airway smooth muscle to airway narrowing and airway hyperresponsiveness in disease. *Eur. Respir. J.* **16**: 349–354.

Mercier, F.J., Naline, E., Bardou, M., Georges, O., Denjean, A., Benhamou, D. and Advenier, C. (2002). Relaxation of proximal and distal isolated human bronchi by halothane, isoflurane and desflurane. *Eur. Respir. J.* **20**: 286–292.

Seow, C.Y., Schellenberg, R.R. and Pare, P.D. (1998). Structural and functional changes in the airway smooth muscle of asthmatic subjects. *Am. J. Respir. Crit. Care Med.* **158**: S179–S186.

Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. and Narumiya, S. (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* **389**: 990–994.

Yamakage, M., Kohro, S., Kawamata, T. and Namiiki, A. (1993). Inhibitory effects of four inhaled anesthetics on canine tracheal smooth muscle contraction and intracellular Ca\(^{2+}\) concentration. *Anesth. Analg.* **77**: 67–72.

Yoshii, A., Iizuka, K., Dobashi, K., Horie, T., Harada, T., Nakazawa, T. and Mori, M. (1999). Relaxation of contracted rabbit tracheal and human bronchial smooth muscle by Y-27632 through inhibition of Ca\(^{2+}\) sensitization. *Am. J. Respir. Cell Mol. Biol.* **20**: 1190–1200.