J Anesth (2008) 22:21–26
DOI 10.1007/s00540-007-0567-1

Journal of
Anesthesia

High concentrations of landiolol, a β_1-adrenoceptor antagonist, stimulate smooth muscle contraction of the rat trachea through the Rho-kinase pathway

Osamu Shibata, Kenji Nishioka, Masakazu Yamaguchi, Tetsuji Makita, and Koji Sumikawa

Department of Anesthesiology, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

Abstract

Purpose. Gradually progressing contraction of airway smooth muscle is suggested to be due to the Rho-kinase signaling pathway. In our preliminary study in rat tracheas, landiolol, a β_1-adrenoceptor antagonist, at high doses caused gradually progressing contraction, and this contraction reached a plateau after 20 min. Therefore, this study was carried out to clarify whether landiolol could stimulate the Rho-kinase pathway or the phosphatidylinositol (PI) response in the rat trachea.

Methods. Seventy-eight male Wistar rats weighing 250–350 g were used for the experiments. Their tracheas were cut into 3-mm-wide ring segments or 1-mm-wide slices. Measurements of isometric tension and [^{3}H] inositol monophosphate (IP_1) production were conducted, using these tracheal rings or slices. Data values are expressed as means ± SD, and statistical significance ($P < 0.05$) was determined using analysis of variance (ANOVA).

Results. Landiolol (700 μM)-induced contraction was completely inhibited by fasudil at 30 μM, while the landiolol-induced contraction was not inhibited by 4-diphenylacetoxy-N-methyl-piperidine methobromide (4-DAMP), ketanserin, or nicardipine. Landiolol did not stimulate IP_1 production.

Conclusion. These results suggest that high concentrations of landiolol could cause airway smooth muscle contraction through the Rho-kinase pathway, but not through the PI response coupled with muscarinic M_3 receptors, 5-HT receptors or the activation of L-type Ca^{2+} channels.

Key words Landiolol · β_1-adrenoceptor antagonist · Rho-kinase pathway · Phosphatidylinositol response · Tracheal smooth muscle

Address correspondence to: O. Shibata
Received: November 6, 2006 / Accepted: August 7, 2007

Introduction

Among the many β_1-selective adrenoceptor antagonists used clinically, several are known to have additional β_2-effects at high doses. One such agent is landiolol, a new potent selective β_1-adrenoceptor antagonist, used in the treatment of tachyarrhythmias [1–3].

In an experiment with rat tracheal rings, we found that contraction induced by acetylcholine (ACh) quickly progressed and reached a plateau within 5 min, while contractions induced by anticholinesterase (anti-ChE) drugs gradually progressed and reach a plateau after 30 min [4]. Although there was no significant difference in strength between the contractions induced by ACh and Anti-ChE drugs, the Anti-ChE -induced contractions were completely inhibited by Rho-kinase inhibitors, while the ACh-induced contraction was inhibited incompletely [4]. These findings suggest that gradually progressing contractions of airway smooth muscle might be due to the Rho-kinase pathway.

Airway smooth muscle contraction is regulated by myosin light chain (MLC) phosphorylation (Fig. 1). When receptors on the airway smooth muscle cell membranes stimulate G_q-proteins to activate phospholipase C, inositol 1,4,5 trisphosphate (IP_3) is increased. Inositol 1,4,5 trisphosphate mobilizes Ca^{2+} from the sarcoplasmic reticulum, and at the same time Ca^{2+} flows inward from the extracellular space, resulting in an increase in intracellular Ca^{2+} concentration. The increase in Ca^{2+} activates MLC kinase, resulting in an increase in MLC phosphorylation. On the other hand, when receptors on the airway smooth muscle stimulate heterotrimeric G-proteins and subsequently Rho (small G-proteins), the Rho-kinase pathway is activated, and then myosin phosphatase is inactivated, resulting in an increase in MLC phosphorylation [5–8].

In our preliminary study, while esmolol did not affect the resting tension of rat tracheal rings, landiolol caused a gradually progressing contraction. This contraction

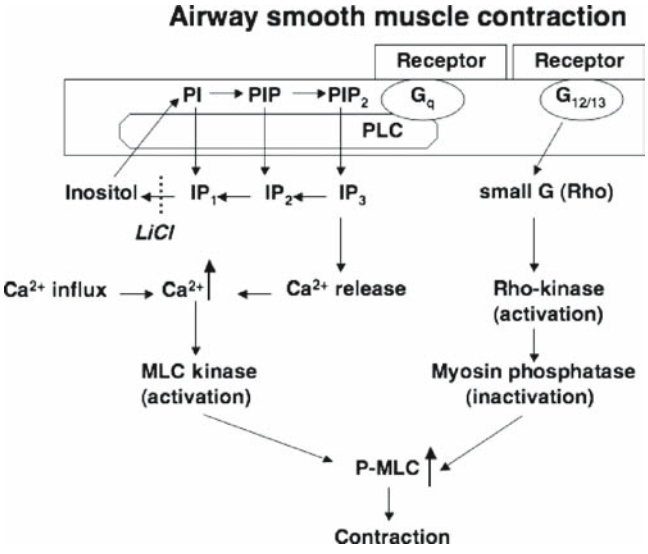

Fig. 1. A flow diagram of the phosphatidylinositol (*PI*) response and Rho-kinase pathway. *G*, G-protein; *PIP*, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5 trisphosphate; IP_2, inositol bisphosphate; IP_1, inositol monophosphate; *MLC*, myosin light chain; *P-MLC*, phosphorylated myosin light chain; *PLC*, phospholipase C

reached a plateau after 20 min, and then was sustained over 90 min. A submaximal dose (700 μM) of landiolol had a potency nearly equal to that of 10 μM acetylcholine to induce the contractile response. The mechanisms involved in the effect of landiolol in causing the contraction of airway smooth muscle are not fully understood. The present study was carried out to clarify the mechanism of action of landiolol, by examining the effects of a Rho-kinase inhibitor, a muscarinic M_3 receptor antagonist, a 5-HT receptor antagonist, and an L-type Ca^{2+} channel blocker.

Materials and methods

This study was conducted following guidelines approved by our Institutional Animal Care Committee. Seventy-eight male Wistar rats (Charles River, Yokohama, Japan) weighing 250–350 g were used for the experiments. The rats were exsanguinated under anesthesia with intraperitoneal pentobarbital (50 $mg \cdot kg^{-1}$ intraperitoneal), and the trachea was rapidly isolated.

Contractile response

Each trachea was cut into 3-mm-wide ring segments with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, UK). We used only the distal three rings of the trachea, i.e., within 9 mm from the carina, because the contractile responses differ between the proximal and distal segments [9]. In each experiment, eight rings from three rats were used in eight organ chambers. The tracheal ring was suspended between two stainless steel hooks and placed in a 5-ml water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit (K-H) solution (mM composition; NaCl, 118; KCl, 4.7; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11; Na_2-ethylenediamine tetraacetic acid [EDTA], 0.05). The solution was continuously aerated with O_2 95%/CO_2 5% at 37°C. Isometric tensions were measured using an isometric transducer (Kishimotoika) and changes in isometric force were recorded using a MacLab system (Milford, MA, USA). The resting tension was periodically adjusted to 1.0 g during the equilibration period. The rings were washed every 15 min and re-equilibrated to baseline tension for 60 min (time 0).

At time 0, landiolol was added stepwise, cumulatively, to induce active contraction at 0 μM to 1000 μM final concentrations. Eight tracheal rings from four rats were used in two experiments.

To examine whether landiolol-induced contraction could be mediated through a muscarinic receptor, a 5-HT receptor, an L-type Ca^{2+} channel, or the Rho-kinase pathway, 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP; a muscarinic M_3 receptor antagonist), ketanserin (a 5-HT receptor antagonist), nicardipine (an L-type Ca^{2+} channel blocker), fasudil (a Rho-kinase inhibitor), or none of them was added stepwise, cumulatively, 30 min after the addition of landiolol at 700 μM (submaximal dose). Thirty-six tracheal rings from 18 rats were used in six experiments.

To examine whether an appropriate concentration of inhibitor could inhibit or reduce the tracheal contraction induced by the cumulative addition of landiolol, a 10-μM final concentration of each of 4-DAMP, ketanserin, nicardipine, fasudil, or none of them was added 15 min before the addition of landiolol. Landiolol was added stepwise, cumulatively, to induce active contraction at 0 μM to 1000 μM final concentrations. Forty tracheal rings from 18 rats were used in seven experiments.

To examine whether the landiolol-induced contraction was attenuated by β-receptor agonists or phosphodiesterase inhibitors, isoproterenol, salbutamol (a β_2-receptor agonist), dobutamine (a β_1-receptor agonist), or aminophylline was added stepwise, cumulatively, 30 min after the addition of landiolol at 700 μM. Thirty-two tracheal rings from 21 rats were used in seven experiments.

To examine whether ACh-induced contraction, which showed nearly equal values to 700-μM landiolol-induced contraction, was attenuated by isoproterenol, isoproter-

enol was added stepwise, cumulatively, 10 min after the addition of 10 μM ACh. Six tracheal rings from three rats were used in one experiment.

PI response

A modified technique of Brown et al. [10] was used. Inositol 1,4,5-trisphosphate (IP_3) is rapidly degraded into inositol monophosphate (IP_1) and subsequently recycled back to phosphatidylinositol (PI) via free inositol. Lithium inhibits the conversion of IP_1 to inositol, and in the presence of Li^+ the production rate of the IP_1 reflects the extent of the PI response. We measured [^{3}H]IP_1 in tracheal slices incubated with [^{3}H]*myo*-inositol (Amersham, Tokyo, Japan). Each trachea was longitudinally cut and chopped into 1-mm-wide slices with a McIlwain tissue chopper. Three tracheal slices were placed in small flat-bottomed tubes and preincubated for 15 min in K-H solution containing 10 mM LiCl and continuously aerated with $O_2$95%/$CO_2$5%. An aliquot of 0.5 μCi [^{3}H]*myo*-inositol was then added to each tube (final concentration of 0.1 μM in a 300-μl incubation volume). The tubes were then flushed with $O_2$95%/$CO_2$5%, capped, set in a shaking bath at 37°C, and incubated for 30 min (time 0).

To examine whether the landiolol-induced contraction would be mediated through the PI response, we measured IP_1 production in the rat tracheal slices. We used 14 rats. Landiolol, 700 μM, carbachol (CCh), 10 μM, or nothing was added at time 0. To support negative findings, a positive control was conducted with CCh, which is known to increase IP_1 production. After 60 min, the reaction was stopped with 940 μl of chloroform: methanol (1: 2 v/v). Chloroform and water were then added (300 μl each) and the phases were separated by centrifugation at 90 *g* for 5 min. The [^{3}H]IP_1 was separated from [^{3}H]*myo*-inositol in the 750-μl water phase by column chromatography, using Dowex AG 1-X8 resin (Bio Rad, Richmond, CA, USA) in its formate form. [^{3}H]IP_1 produced in the tracheal slices was measured using a liquid scintillation counter, and values are presented as disintegration per minute (DPM). The scintillation counts for the blank values (no slices present) were subtracted to obtain the experimental data.

Data values are expressed as means ± SD. The results were subjected to one-way analysis of variance followed by Scheffe's F-test. A value of $P < 0.05$ was considered statistically significant.

Results

A recording of landiolol-induced contraction in a rat tracheal ring is shown in Fig. 2A. Figure 2B shows the effects of landiolol on resting tension in rat tracheal rings. Figure 3A shows the effects of 4-DAMP, ketanserin, fasudil, nicardipine, or none of them, on landiolol (submaximal dose, 700 μM)-induced tension in rat tracheal rings. Landiolol-induced contraction was not inhibited by either 4-DAMP, ketanserin, or nicardipine. Landiolol-induced contraction was completely inhibited by fasudil at a dose of 30 μM. The ID_{50} value for the effect of fasudil on landiolol-induced tracheal contraction was 4.0 ± 1.8 μM. Figure 3B shows the effects of the above compounds (10 μM each) on the tracheal contraction induced by the cumulative addition of landiolol. None of these compounds, except for fasudil, inhibited the landiolol-induced contraction. Figure 4 shows the effects of isoproterenol, salbutamol, aminophylline, and dobutamine on the landiolol (700 μM)-induced contraction in rat tracheal rings. The ID_{50} value for the effect of isoproterenol on landiolol-induced tracheal contraction was 0.70 ± 0.38 μM. Landiolol-induced contraction

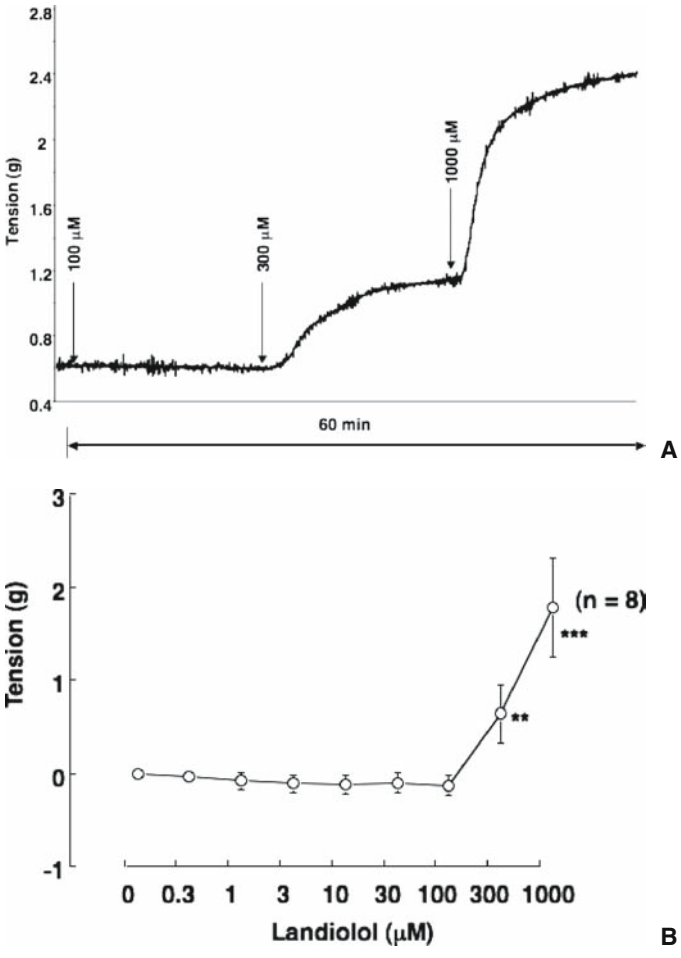

Fig. 2. A Recording of landiolol-induced contraction in a rat tracheal ring. **B** Effects of landiolol on resting tension in rat tracheal rings (mean ± SD). $^{**}P < 0.01$; $^{***}P < 0.001$ vs landiolol 0

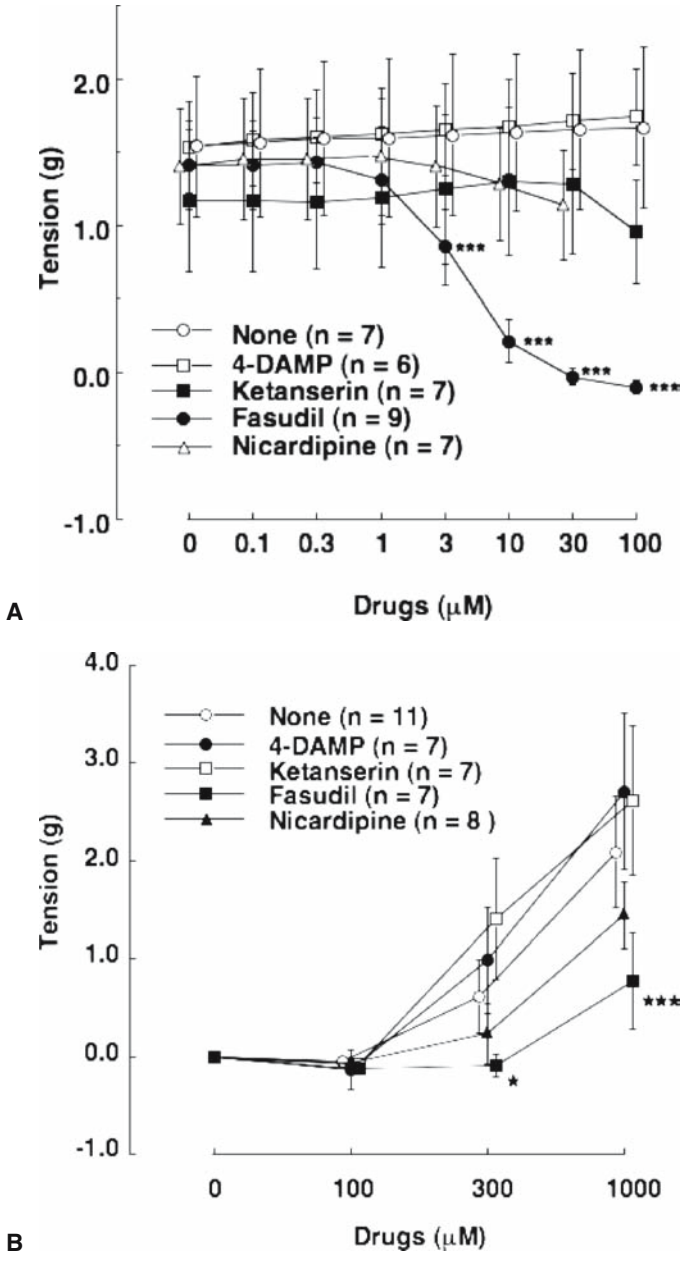

Fig. 3. **A** The effects of 4-diphenylacetoxy-N-methyl piperidine methobromide (*4-DAMP*; a muscarinic M_3 receptor antagonist), ketanserin (a 5-HT receptor antagonist), fasudil (a Rho-kinase inhibitor) and nicardipine (an L-type Ca^{2+} channel blocker), or none of them, on landiolol-induced tension in rat tracheal rings (mean ± SD). Landiolol; 700 μM. ***$P < 0.001$ vs fasudil 0. **B** The effects of 4-DAMP (a muscarinic M_3 receptor antagonist), ketanserin (a 5-HT receptor antagonist), fasudil (a Rho-kinase inhibitor), nicardipine (an L-type Ca^{2+} channel blocker), or none of them, on the stepwise cumulative addition of landiolol (mean ± SD). All drugs, 10 μM. *$P < 0.05$; ***$P < 0.001$ vs none

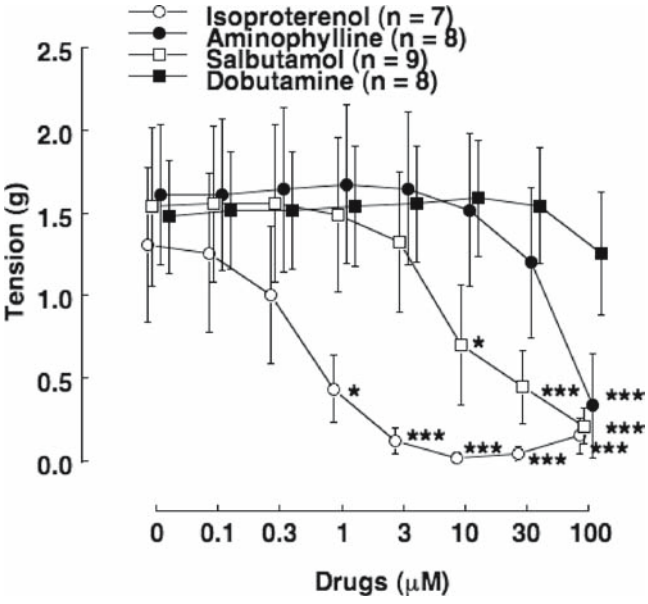

Fig. 4. The effects of isoproterenol (a β-receptor agonist), salbutamol (a β_2-receptor agonist), dobutamine (a β_1-receptor agonist), and aminophylline (a phosphodiesterase inhibitor) on landiolol-induced contraction in rat tracheal rings (mean ± SD). Landiolol, 700 μM. *$P < 0.05$, ***$P < 0.001$ vs drugs 0

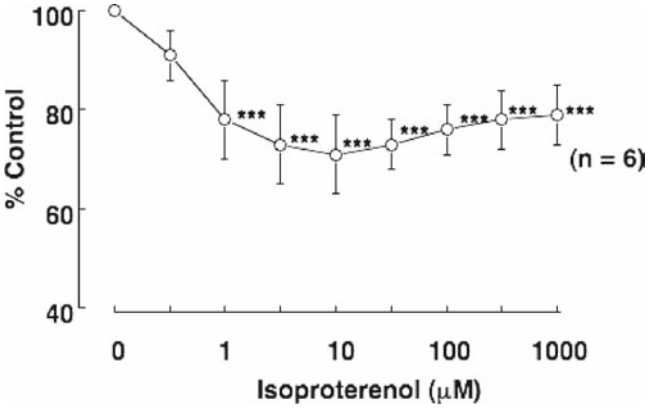

Fig. 5. The effects of isoproterenol on acetylcholine (ACh)-induced contraction in rat tracheal rings (mean ± SD). ACh, 10 μM. ***$P < 0.001$ vs isoproterenol 0

was 86% inhibited by salbutamol and 79% inhibited by aminophylline (each at 100 μM), while the landiolol-induced contraction was not inhibited by dobutamine. The ACh-induced contraction, which showed values nearly equal to the values for landiolol (700 μM)-induced contraction, was attenuated by 29% by isoproterenol (Fig. 5), while the landiolol-induced contraction was completely inhibited by isoproterenol at a dose of 10 μM (Fig. 4). The effects of landiolol and CCh on IP_1 production in rat tracheal slices are shown in Fig. 6. Carbachol, but not landiolol, increased IP_1 production.

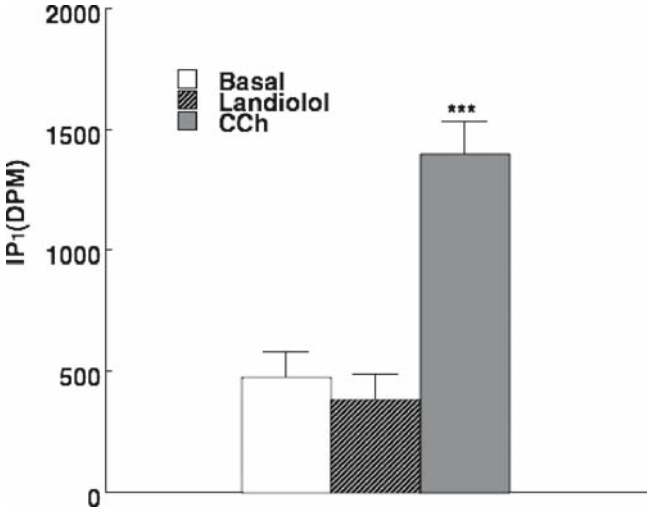

Fig. 6. The effects of landiolol on IP_1 production in rat tracheal slices (mean ± SD). *IP_1*, inositol monophosphate; *DPM*, disintegration per minute. Landiolol, 700 μM, carbachol (*CCh*), 10 μM. ***$P < 0.001$ vs basal or landiolol

Discussion

The present results show that landiolol induces contraction of the rat trachea, and that this contraction is completely abolished by fasudil, but not inhibited by 4-DAMP, ketanserin, or nicardipine. The results also show that landiolol does not stimulate a PI response, while it induces the contraction of rat trachea.

When agonists stimulate receptors on airway smooth muscle cell membranes, G_q- and heterotrimeric G-proteins activate the PI response and the Rho-kinase pathway, respectively, resulting in airway smooth muscle contractions (Fig. 1). Possible mechanisms involved in the landiolol-induced contraction are as follows. The airway smooth muscle contraction occurs through the activation of receptors coupled with small G-proteins in canine, rabbit, and human airway smooth muscles in vitro, and involves the Rho-kinase pathway [5–8]. Rho, a small G-protein, activates Rho-kinase, which in turn inactivates myosin phosphatase. Inactivation of myosin phosphatase increases myosin light chain (MLC) phosphorylation, resulting in an increased contraction. In the present study, we examined the role of the Rho-kinase pathway in the effects of landiolol, and found that fasudil completely abolished the landiolol-induced contraction of the rat trachea. Thus, it is possible that landiolol activates the upstream axis of Rho kinase activation, including $G_{12/13}$ receptors and Rho A, as well as Rho kinase itself. In contrast to the above mechanism, landiolol may stimulate ACh release from postganglionic parasympathetic nerve endings, which in turn activate a receptor-coupled PI response, resulting in airway smooth muscle contraction. Janssen et al. [11] examined prejunctional β-adrenoceptors in canine bronchi by observing the effects of β-receptor antagonists on field stimulation-induced contractions, and they concluded that catecholamines acted on prejunctional β-receptors, resulting in inhibition of cholinergic neurotransmission in the canine bronchi. Landiolol may inhibit prejunctional β-receptors, resulting in the activation of cholinergic neurotransmission, and subsequent stimulation of the contractile response in the rat trachea. However, in the present study, landiolol-induced contraction was not inhibited by 4-DAMP, a muscarinic M_3 receptor antagonist. Thus, the landiolol-induced contraction was not mediated by ACh.

ACh-induced contraction, which showed values nearly equal to those for landiolol-induced contraction, was attenuated by 29% by isoproterenol, while the landiolol-induced contraction was completely inhibited by isoproterenol. Oguma et al. [12] measured tension and intracellular Ca^{2+} in guinea-pig tracheal smooth muscles stimulated by methacholine, and found that, in isoproterenol-induced relaxation, the reduction in tension was greater than the reduction in intracellular Ca^{2+}. They concluded that β-adrenergic action would antagonize not only Ca^{2+} mobilization but also Ca^{2+} sensitization. Ca^{2+} mobilization is mediated through the PI response, while Ca^{2+} sensitization would be mediated through Rho-kinase signaling. ACh-induced contraction is mediated through both the PI response and Rho-kinase signaling, while the landiolol-induced contraction would be mediated only through Rho-kinase signaling. Thus, this might be a reason why the landiolol-induced contraction was completely inhibited by isoproterenol.

In the present study, the effective concentrations of landiolol for inducing contraction in rat tracheal rings was 300 μM ($P < 0.01$). The peak serum concentration of landiolol is approximately 1000 ng·ml^{-1} (1.8 μM) in clinical settings [13]. The concentrations used in the present study are two orders of magnitude higher than the clinically relevant concentrations. Thus, our results suggest that landiolol would not induce bronchoconstriction in clinical settings.

In the present study, landiolol did, but esmolol did not, have effects on tracheal tension. Although esmolol and landiolol are very similar pharmaceutically and pharmacologically, their actions on trachea are different. Esmolol is a racemic mixture, while landiolol is an optical isomer. This difference may underlie the differences in their actions on the trachea.

In conclusion, high concentrations of landiolol could cause airway smooth muscle contraction through activation of the Rho-kinase pathway.

Acknowledgments. This study was supported in part by Grant 19591804 for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

References

1. Kinoshita H, Kakutani T, Mizumoto K, Hatano Y (2005) Effectiveness of bolus landiolol on paroxysmal atrial tachycardia. Can J Anaesth 52:999–1000
2. Yoshida Y, Hongo T, Sakamoto A, Ogawa R (2005) Successful management of tachycardiac atrial fibrillation in a septic patient with landiolol. Anesth Analg 100:294
3. Nishina K, Mikawa K, Yonemoto Y, Sugimoto Y (2003) The efficacy of bolus administration of landiolol for attenuating tachycardia in pheochromocytoma. Anesth Analg 97:294–295
4. Shibata O, Saito M, Yoshimura M, Yamaguchi M, Nishioka K, Makita T, Sumikawa K (2006) Anticholinesterase drugs stimulate smooth muscle contraction of the rat trachea through the Rho-kinase pathway. Anesth Analg 102:1121–1126
5. Iizuka K, Dobashi K, Yoshii A, Horie T, Suzuki H, Nakazawa T, Mori M (1997) Receptor-dependent G protein-mediated Ca^{2+} sensitization in canine airway smooth muscle. Cell Calcium 22: 21–30
6. Iizuka K, Yoshii A, Samizo K, Tsukagoshi H, Ishizuka T, Dobashi K, Nakazawa T, Mori M (1999) A major role for the rho-associated coiled coil forming protein kinase in G-protein-mediated Ca^{2+} sensitization through inhibition of myosin phosphatase in rabbit trachea. Br J Pharmacol 128:925–933
7. Yoshii A, Iizuka K, Dobashi K, Horie T, Harada T, Nakazawa T, 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
8. Iizuka K, Shimizu Y, Tsukagoshi H, Yoshii A, Harada T, Dobashi K, Murozono T, Nakazawa T, Mori M (2000) Evaluation of Y-27632, a rho-kinase inhibitor, as a bronchodilator in guinea pigs. Eur J Pharmacol 406:273–279
9. de Lima WT, da Silva ZL (1998) Contractile responses of proximal and distal trachea segments isolated from rats subjected to immunological stimulation: role of connective tissue mast cells. Gen Pharmacol 30:689–695
10. Brown E, Kendall DA, Nahorski SR (1984) Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. Receptor characterization. J Neurochem 42:1379–1387
11. Janssen LJ, Tazzeo T, Zuo J (2004) Enhanced myosin phosphatase and Ca^{2+}-uptake mediate adrenergic relaxation of airway smooth muscle. Am J Respir Cell Mol Biol 30:548–554
12. Oguma T, Kume H, Ito S, Takeda N, Honjo H, Kodama I, Shimokata K, Kamiya K (2006) Involvement of reduced sensitivity to Ca^{2+} in beta-adrenergic action on airway smooth muscle. Clin Exp Allergy 36:183–191
13. Nakashima M, Kanamaru M (2000) Phase I study of ONO-1101, a new ultra short acting β_1-blocking agent in healthy volunteers. Rinsyo Iyaku (J Clinical Therapeutics & Medicines) 16:1531–1556