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Lidocaine attenuates muscarinic receptor-mediated inhibition of adenylyl cyclase in airway smooth muscle

Motonari Yunoki, Tsutomu Nakahara*, Akiko Mitani, Takeshi Maruko, Yuko Kubota, Kenji Sakamoto, Kunio Ishii

Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

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Abstract

We examined how lidocaine affects muscarinic receptor-mediated inhibition of adenylyl cyclase in bovine tracheal smooth muscles. Lidocaine (100 μM) augmented the relaxant responses to forskolin in the bovine tracheal smooth muscle contracted with methacholine (0.3 μM). On the other hand, lidocaine failed to affect the relaxant effects of forskolin on the histamine (100 μM)- and KCl (40 mM)-contracted preparations. Lidocaine (100 μM) enhanced both basal and forskolin-stimulated cAMP accumulation in the presence of methacholine (0.3 μM). However, in the absence of methacholine, neither basal nor forskolin-stimulated cAMP accumulation was affected by lidocaine. Similar phenomenon was observed when the bovine tracheal smooth muscles were treated with methoctramine (0.03 μM). In radioligand binding experiments, lidocaine inhibited [^{3}H]*N*-methyl scopolamine binding to cloned human muscarinic receptors (M_1–M_5) expressed in Chinese hamster ovary cells. These results suggest that lidocaine prevents muscarinic receptor-mediated signaling pathway and thereby reverses inhibition of adenylyl cyclase by methacholine in bovine tracheal smooth muscle.

Keywords: cAMP; (Bovine); Lidocaine; Anaesthetic, local; Smooth muscle

1. Introduction

Lidocaine is administered to prevent bronchospasm associated with airway instrumentation for general anaesthesia or bronchoscopy. Not only attenuation of vagal reflex arcs (Downes et al., 1980; Brown et al., 1995; Groeben et al., 1996) and inhibition of chemical mediator release (Weiss et al., 1978), but also the relaxing effect on airway smooth muscle (Weiss et al., 1975; Downes and Loehning, 1977; Okumura and Denborough, 1980; Kai et al., 1993) could contribute to the prevention of bronchospasm. Recently, we have shown that lidocaine augmented that relaxant responses to adenosine 3′,5′-cyclic monophosphate (cAMP)-elevating drugs, such as salbutamol and forskolin, in bovine tracheal smooth muscle contracted with methacholine (Nakahara et al., 2000). However, the mechanism of that augmentation remains to be elucidated.

In bovine tracheal smooth muscle, the expression of at least two muscarinic receptor subtypes M_2 and M_3 has been established (Roffel et al., 1987; Lucchesi et al., 1990). Stimulation of muscarinic M_2 receptors decreases adenylyl cyclase activity via a G_i protein, thereby lowering cAMP levels (Sankary et al., 1988; Yang et al., 1991; Schaefer et al., 1995). On the other hand, stimulation of muscarinic M_3 receptors causes phosphoinositide hydrolysis via a G_q protein, thereby increasing production of inositol 1,4,5-trisphosphate and diacylglycerol (Roffel et al., 1990). Thus, muscarinic M_2 receptors contribute to negative regulation of intracellular cAMP levels and muscarinic M_3 receptors play an important role in the development of airway contraction. If lidocaine prevents muscarinic M_2 receptor-mediated signaling pathways in bovine tracheal smooth muscle contracted with methacholine, then lidocaine augments the relaxant effects of cAMP-elevating drugs. The purpose of this study, therefore, was to examine how lidocaine affects muscarinic receptor-mediated inhibition of adenylyl cyclase in bovine tracheal smooth muscles.

* Corresponding author. Fax: +81-3-3444-6205.
E-mail address: nakaharat@pharm.kitasato-u.ac.jp (T. Nakahara).

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2. Materials and methods

2.1. Preparation of bovine tracheal smooth muscle segments

Fresh bovine tracheas were obtained from a local abattoir and transported to the laboratory in icecold Krebs–Ringer bicarbonate buffer (composition in mM: NaCl 118.5, KCl 4.47, $MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 2.54, $NaHCO_3$ 24.9, glucose 10.0, pyruvic acid 1.0) (pH = 7.4). The smooth muscle layers were dissected from the cartilage, mucosa and connective tissues while immersed in icecold Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 as described previously (Nakahara et al., 2000). Segments ($1 \times 2 \times 10$ mm) of smooth muscle were used for measurement of mechanical responses and of cAMP content.

2.2. Measurement of mechanical activity

One end of each muscle was attached to an isometric force displacement transducer (model TB-611T, Nihon Kohden, Tokyo, Japan) by a cotton thread, and the other end was tied to a stainless steel holder. The muscle segments were mounted in 20-ml jacketed organ baths containing Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2 at 37 °C and subsequently allowed to equilibrate for 1 h under an initial tension of 0.75 g. The bath solution was changed every 15 min during the incubation period. The resting tension was adjusted to 0.5 g 10 min before starting each experiment.

When plateau tone was reached 15 min after the addition of methacholine (0.3 μM), tissues were exposed to vehicle (Krebs–Ringer bicarbonate buffer), lidocaine (100 μM) or methoctramine (0.03 μM). After an additional 15-min incubation period, the tissues were relaxed by the addition of forskolin (0.001–10 μM) or 8-(4-chlorophenylthio)adenosine 3′, 5′-cyclic monophosphate (8-CPT-cAMP)(1–300 μM). We also examined the effect of lidocaine on relaxant responses to forskolin in the preparations contracted with 100 μM histamine or 40 mM KCl. In experiments using high K^+ solution, Na^+ in the bathing medium was replaced by an equimolar concentration of K^+. Only one concentration–response curve was constructed for each preparation.

2.3. Measurement of cAMP content

To measure cAMP accumulation, all experiments were conducted in the presence of phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (300 μM). The effect of lidocaine on changes in tissue content of cAMP induced by forskolin (1 μM) was examined in the presence and absence of methacholine (0.3 μM). Each tissue was equilibrated for 60 min in an organ chamber that was warmed to 37 °C and filled with 5 ml of Krebs–Ringer bicarbonate buffer gassed with 95% O_2–5% CO_2. After the equilibration period, tissues were exposed to 3-isobutyl-1-methylxanthine and either methacholine or vehicle (Krebs–Ringer bicarbonate buffer). Then, the tissues were treated with vehicle, lidocaine (100 μM) or methoctramine (0.03 μM) 15 min after the additions of 3-isobutyl-1-methylxanthine and either methacholine or vehicle. After an additional 15-min incubation period, the tissues were incubated for 10 min with forskolin or vehicle (dimethyl sulfoxide, DMSO). At the end of the incubation period, the tissues were rapidly frozen in liquid nitrogen and stored at −80 °C until homogenization in 2 ml of icecold 6% trichloroacetic acid, using a glass homogenizer. The homogenate was centrifuged at $1500 \times g$ for 10 min at 4 °C. The supernatant was extracted three times with 5 ml of diethyl ether. The cAMP content was determined using a method of radioimmunoassay (cAMP assay kit, Yamasa Shoyu, Choshi, Japan). The tissue residue was dissolved in 2 N NaOH and the protein content was determined using a protein assay kit (Bio-Rad protein Assay, Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin as the standard. The tissues content of cAMP was presented as pmol/mg protein.

2.4. Cloned cell culture

Five cloned human muscarinic receptors (M_1–M_5) stably expressed in Chinese hamster ovary (CHO) cells were obtained from Public Health Service (National Institutes of Health, Rockville, MD, USA). These cells were prepared by Drs. T.I. Bonner and N.J. Buckley (National Institute of Mental Health, Bethesda, MD, USA) and Dr. M.R. Brann (National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA). Cells were incubated at 37 °C in a humidified atmosphere (5% CO_2) as a monolayer culture in Ham's F-12 Nutrient mixture supplemented with 10% fetal calf serum.

2.5. Radioligand receptor binding assay

Preparation of membranes and radioligand receptor binding studies were conducted as described previously (Kubota et al., 2002). The K_d values of muscarinic M_1, M_2, M_3, M_4 and M_5 receptors for [^{3}H]*N*-methyl scopolamine were 383.7 ± 49.2, 295.6 ± 20.4, 242.7 ± 16.2, 448.5 ± 71.0 and 374.1 ± 23.6 pM, respectively, and the B_{max} of muscarinic M_1, M_2, M_3, M_4 and M_5 receptors were 360.6 ± 36.3, 158.0 ± 8.5, 459.2 ± 14.5, 28.9 ± 2.5 and 92.6 ± 6.3 fmol/mg protein, respectively (Kubota et al., 2002). Competition binding studies were carried out using a fixed concentration (143 pM) of [^{3}H]*N*-methyl scopolamine (70 Ci/mmol; NEN Life Science Products) in the absence and presence of lidocaine (1–1000 μM). Reactions were terminated by rapid filtration through Whatman GF/C glass filters (Whatman, Kent, UK) using a Brandel cell harvester (model M-12; Brandel, Gaithersburg, MD, USA). The filters were washed three times with the icecold buffer.

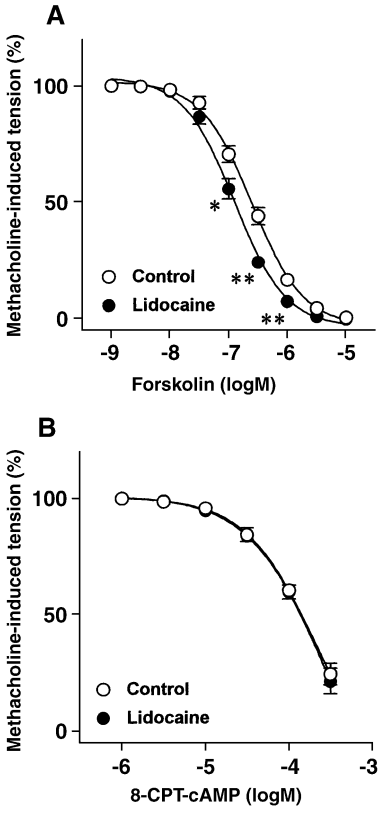

Fig. 1. Effect of lidocaine (100 μM) on the relaxant responses to forskolin (A) and 8-CPT-cAMP (B) in the bovine tracheal smooth muscles contracted with methacholine (0.3 μM). Data are expressed as the percentage change in methacholine-induced tension measured just before the cumulative addition of forskolin or 8-CPT-cAMP. (A) The methacholine-induced tensions in the absence and presence of lidocaine were 9.0 ± 0.7 g ($n=7$) and 7.6 ± 0.5 g ($n=7$), respectively. (B) The methacholine-induced tensions in the absence and presence of lidocaine were 8.6 ± 0.7 g ($n=4$) and 5.4 ± 0.8 g ($n=4$), respectively. Each point with a vertical bar represents mean ± S.E.M. from four to seven separate preparations. $^{*}P<0.05$, $^{**}P<0.01$ vs. corresponding control values.

They were placed in liquid scintillation counting vials containing 4 ml of Clear-sol I (Nacalai Tesque, Kyoto, Japan). Radioactivity was measured in a liquid scintillation counter (LS6000 IC; Beckman Instruments, CA, USA). The specific binding of [^{3}H]*N*-methyl scopolamine was defined as the total binding minus the nonspecific binding which was determined in the presence of 1 μM atropine.

2.6. Data analysis and statistics

Relaxant responses were expressed as percentages of 0.3 μM methacholine-, 100 μM histamine- or 40 mM K^+-induced tension obtained just before the cumulative addition of drugs. The half-maximum effective concentration values (EC_{50}) of forskolin were calculated using the GraphPad Prism™ program (GraphPad Software, San Diego, CA, USA). The pD_2 values were calculated as the negative logarithm of EC_{50}.

Competition binding curves were analyzed using the GraphPad Prism™ program (GraphPad Software). The equilibrium constant (K_d) and the maximum binding (B_{max}) were calculated from Scatchard analysis. The affinity of lidocaine for receptors in competition for radioligand binding (K_i) was determined by the method of Cheng and Prusoff (1973).

Data were expressed as the means ± S.E.M. and were analyzed using either Student's *t*-test or Dunnett's or Tukey's multiple comparison test after one-way analysis of variance. A *P* value smaller than 0.05 was considered significant.

2.7. Drugs

The following drugs were used: acetyl-β-methylcholine chloride (methacholine), 8-(4-chlorophenylthio)adenosine 3′, 5′-cyclic monophosphate (8-CPT-cAMP), forskolin, histamine dihydrochloride, 3-isobutyl-1-methylxanthine, lidocaine hydrochloride, methoctramine tetrahydrochloride (Sigma, St. Louis, MO, USA).

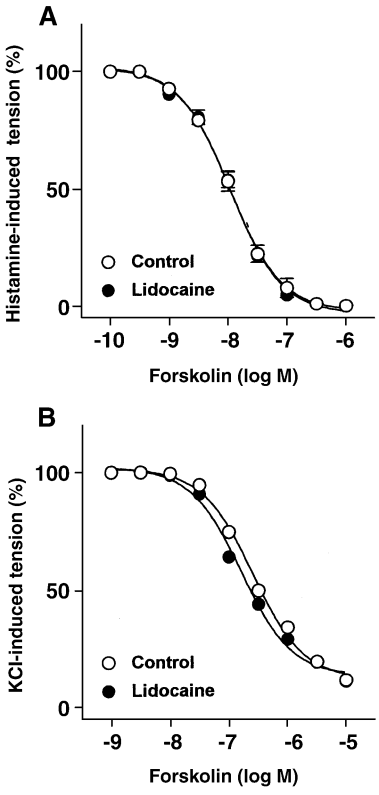

Fig. 2. Effect of lidocaine (100 μM) on the relaxant responses to forskolin in the bovine tracheal smooth muscles contracted with histamine (100 μM) (A) and KCl (40 mM) (B). Data are expressed as the percentage change in histamine- or KCl-induced tension measured just before the cumulative addition of forskolin. Each point with a vertical bar represents mean ± S.E.M. from four separate preparations.

3. Results

3.1. Effect of lidocaine on relaxant responses to forskolin and 8-CPT-cAMP in the preparations contracted with methacholine

Lidocaine (100 μM) attenuated the methacholine (0.3 μM)-induced tensions from 8.7 ± 0.5 to 6.2 ± 0.6 g ($P < 0.01$, $n = 11$) and shifted the concentration–response curves for forskolin-induced relaxations to the left (pD_2; control, 6.61 ± 0.03 vs. lidocaine, 6.90 ± 0.03, $P < 0.01$, $n = 7$) (Fig. 1A). However, lidocaine did not affect the relaxant responses to 8-CPT-cAMP (Fig. 1B).

3.2. Effect of lidocaine on relaxant responses to forskolin in the preparations contracted with histamine or high KCl

Tensions in the absence and presence of lidocaine (100 μM) in the preparations contracted with 100 μM histamine were 7.4 ± 0.4 and 7.0 ± 0.3 g, respectively ($n = 4$). On the other hand, lidocaine significantly ($P < 0.05$) attenuated the 40 mM KCl-induced contractions from 11.1 ± 0.5 to 9.4 ± 0.6 g ($n = 4$).

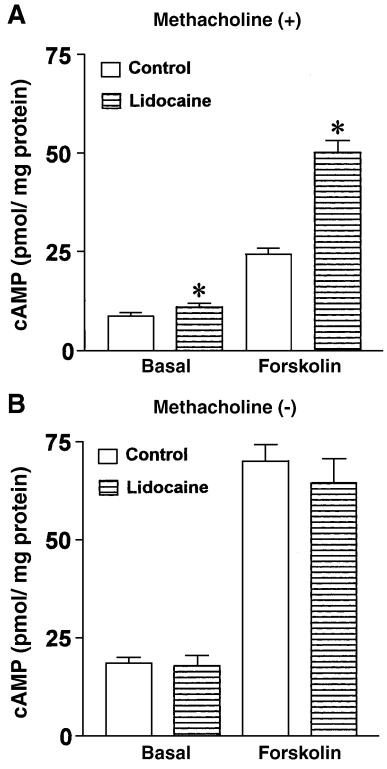

Fig. 3. Effect of lidocaine (100 μM) on basal and forskolin (1 μM)-stimulated cAMP accumulation in the presence (A) and absence (B) of methacholine (0.3 μM). The bovine tracheal smooth muscle preparations were treated with 3-isobutyl-1-methylxanthine (300 μM). Each column with a vertical bar represents mean ± S.E.M. from eight separate preparations. *$P < 0.05$ vs. corresponding control values.

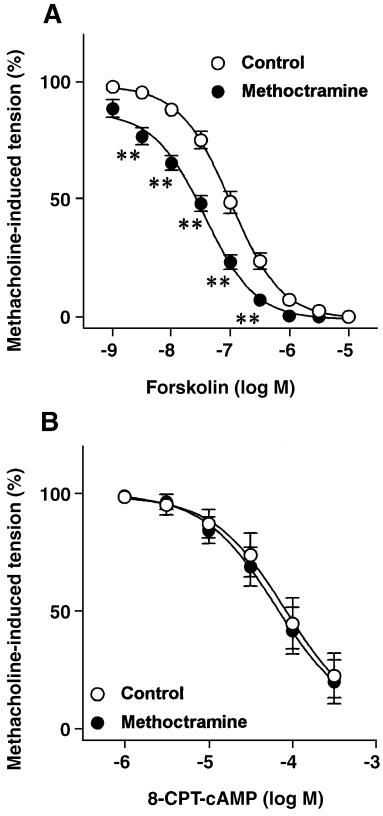

Fig. 4. Effect of methoctramine (0.03 μM) on the relaxant responses to forskolin (A) and 8-CPT-cAMP (B) in the bovine tracheal smooth muscles contracted with methacholine (0.3 μM). Data are expressed as the percentage change in methacholine-induced tension measured just before the cumulative addition of forskolin or 8-CPT-cAMP. Each point with a vertical bar represents mean ± S.E.M. from four to five separate preparations. **$P < 0.01$ vs. corresponding control values.

As shown in Fig. 2, lidocaine did not affect the relaxant responses to forskolin on the preparations contracted with histamine or high KCl.

3.3. Effect of lidocaine on basal and forskolin-stimulated cAMP accumulation

Fig. 3 shows the effect of lidocaine on basal and forskolin-stimulated cAMP accumulation in the presence and absence of methacholine (0.3 μM). In the presence of methacholine, lidocaine enhanced both basal and forskolin-stimulated cAMP accumulation. In contrast, neither basal nor forskolin-stimulated cAMP accumulation was affected by lidocaine in the absence of methacholine.

3.4. Effect of methoctramine on relaxant responses to forskolin and 8-CPT-cAMP in the preparations contracted with methacholine

Methoctramine (0.03 μM) did not change the methacholine-induced tensions (7.3 ± 0.8 vs. 7.2 ± 0.8 g, $n = 9$).

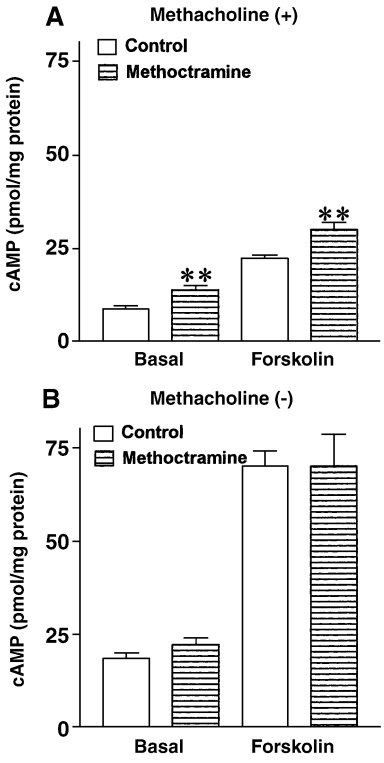

Fig. 5. Effect of methoctramine (100 μM) on basal and forskolin (1 μM)-stimulated cAMP accumulation in the presence (A) and absence (B) of methacholine (0.3 μM). The bovine tracheal smooth muscle preparations were treated with 3-isobutyl-1-methylxanthine (300 μM). Each column with a vertical bar represents mean ± S.E.M. from eight separate preparations. $^{**}P<0.01$ vs. corresponding control values.

Like lidocaine, methoctramine augmented the relaxant responses to forskolin without changing the responses to 8-CPT-cAMP in the preparations contracted with methacholine (Fig. 4).

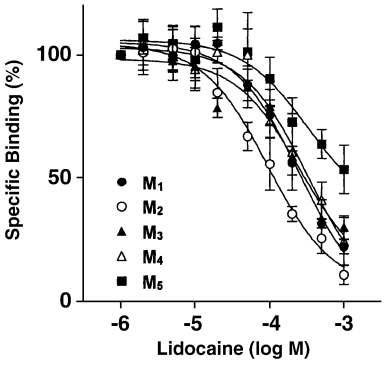

Fig. 6. Effect of lidocaine on the specific binding of [^{3}H]*N*-methyl scopolamine to the membrane fractions prepared from Chinese hamster ovary cells stably expressing human muscarinic receptors (M_1–M_5). Membrane fractions were incubated with a fixed concentration of [^{3}H]*N*-methyl scoplamine (143 pM) in the presence of various concentrations of lidocaine (1–1000 μM) for 60 min. Each point with a vertical bar represents the mean ± S.E.M. from five separate preparations.

Table 1
The $-\log K_i$ value of lidocaine in radioligand binding assays at recombinant human muscarinic receptor subtypes expressed in Chinese hamster ovary cells

M_1	M_2	M_3	M_4	M_5
3.75 ± 0.06	4.22 ± 0.09	3.65 ± 0.08	3.57 ± 0.13	3.33 ± 0.04

Membrane fractions were incubated with a fixed concentration of [^{3}H]*N*-methyl scopolamine (143 pM) in the presence of various concentrations of lidocaine (1–1000 μM) for 60 min. The values represent the means ± S.E.M. from five individual experiments.

3.5. Effect of methoctramine on basal and forskolin-stimulated cAMP accumulation

Fig. 5 shows the effect of methoctramine on basal and forskolin-stimulated cAMP levels in the absence and presence of methacholine (0.3 μM). Methoctramine enhanced both basal and forskolin-induced cAMP accumulation in the presence of methacholine, whereas it failed to affect them in the absence of methacholine.

3.6. Effect of lidocaine on the binding of [^{3}H]N-methyl scopolamine

Lidocaine inhibited the specific [^{3}H]*N*-methyl scopolamine binding to human muscarinic receptors (M_1–M_5) in a concentration-dependent manner (Fig. 6). Table 1 shows the $-\log K_i$ values for inhibition of the specific binding of [^{3}H]*N*-methyl scopolamine to cloned human muscarinic receptors expressed in CHO cells. The $-\log K_i$ value for muscarinic M_2 receptors was significantly ($P<0.05$) larger than those for the other muscarinic receptors. Thus, lidocaine exhibited moderate selectivity for inhibition of muscarinic M_2 receptors.

4. Discussion

Lidocaine augmented the forskolin-induced relaxations of bovine tracheal smooth muscles contracted with methacholine (Nakahara et al., 2000). However, the present study demonstrated that, in the preparations contracted with histamine or high KCl, lidocaine failed to affect the relaxant responses to forskolin. Similarly, the lidocaine-induced potentiation of cAMP accumulation was observed only when tracheal smooth muscle preparation was exposed to methacholine. Our results also indicated that lidocaine displaced the binding of [^{3}H]*N*-methyl scopolamine to human muscarinic receptors. These results suggest that lidocaine prevents muscarinic receptor-mediated signaling pathway and reverses the inhibition of adenylyl cyclase by muscarinic receptor agonists in the bovine tracheal smooth muscles.

The tracheal smooth muscle is known to express at least two subtypes of muscarinic receptors, M_2 and M_3 (Roffel et al., 1987; Lucchesi et al., 1990). Muscarinic M_2 receptors

mediate inhibition of adenylyl cyclase through the pertussis toxin-sensitive G_i protein and muscarinic M_3 receptors mediate activation of phospholipase C-β via a G_q protein (Sankary et al. 1988; Roffel et al. 1990; Yang et al. 1991; Schaefer et al. 1995). Therefore, if lidocaine blocks signaling pathways mediated by muscarinic M_2 receptors, then lidocaine attenuates the inhibition of adenylyl cyclase induced by muscarinic receptor agonists. Indeed, the previous studies have shown that the prevention of muscarinic M_2 receptor-mediated signaling pathway augmented the relaxant responses to cAMP-elevating agents in canine (Fernandes et al., 1992; Mitchell et al., 1993) and guinea pig (Watson and Eglen, 1994) tracheal smooth muscles. Similarly, we also demonstrated that methoctramine (0.03 μM), which neither inhibited the binding of [^{3}H]*N*-methyl scopolamine to muscarinic M_3 receptors (data not shown) nor affected the tensions developed with methacholine, augmented forskolin-induced relaxations without changing the responses to 8-CPT-cAMP in methacholine-contracted bovine tracheal smooth muscle. In addition, our data indicated that lidocaine exhibited moderate selectivity for inhibition of M_2 over the other subtypes of the muscarinic receptors. Ostrom and Ehlert (1998) demonstrated that muscarinic M_2 receptors functionally antagonize the relaxant actions of forskolin, but not isoproterenol in bovine tracheal smooth muscle. They proposed that the relaxant response to isoproterenol appears to be largely independent of cAMP in bovine trachea, and this explains the inability of muscarinic M_2 receptor to inhibit its relaxant effects (Ostrom and Ehlert, 1998). Lidocaine enhanced both relaxant responses to salbutamol and forskolin in bovine tracheal smooth muscle; however, the effect of lidocaine on the forskolin-induced responses was larger than that on salbutamol-induced response (Nakahara et al., 2000). Thus, their results strongly support the idea that lidocaine prevents the signaling pathways elicited by stimulation of muscarinic M_2 receptors.

Many investigators demonstrated that lidocaine has the relaxant action on smooth muscle preparations, including airway smooth muscle (Downes and Loehning, 1977; Okumura and Denborough, 1980; Kai et al., 1993; Fernández del Pozo et al., 1997). We have reported that lidocaine relaxed the bovine tracheal smooth muscles and porcine coronary arteries (Nakahara et al., 2000; Tanaka et al., 2002). The relaxant responses to lidocaine are partly mediated by the inhibition of Ca^{2+} influx (Tanaka et al., 2002). The present results suggest that prevention of muscarinic M_3 receptor-mediated signaling pathway also contributes to the lidocaine-induced relaxations of preparations contracted with methacholine. The decreased levels of precontraction might affect the relaxant responses to bronchodilators; however, lidocaine did not affect the relaxation induced by 8-CPT-cAMP, a membrane-permeable cAMP analogue. Therefore, it is unlikely that the attenuation of precontraction by lidocaine affects cAMP-mediated relaxation of bovine tracheal smooth muscle. Moreover, because diltiazem, a Ca^{2+} channel blocker, could not affect salbutamol- and forskolin-induced relaxation of bovine tracheal smooth muscle (unpublished data), the augmentation by lidocaine of relaxant responses to these drugs cannot be attributed to the inhibitory effect on Ca^{2+} channel.

The preventive effect of lidocaine appears to be mediated by affecting the receptors, the associated G proteins or coupling between them (Xiong et al., 1999; Hollmann et al., 2000, 2001a,b). However, in the present study, lidocaine failed to enhance the relaxant effect of forskolin on histamine-contracted preparations. Thus, it is unlikely that lidocaine nonspecifically uncouples G proteins from the receptors in bovine tracheal smooth muscle. Although the mechanism by which lidocaine prevents muscarinic receptor-mediated signaling pathway is not fully understood, our data suggest that lidocaine might inhibit the binding of muscarinic agonists to muscarinic receptors.

The blood concentration of lidocaine clinically used in the treatment of arrhythmias is 1.5–5 μg/ml (that is, 5.6–19 μM). A lidocaine concentration that produced an apparent synergistic effect on tracheal smooth muscle in this study was much higher than the blood concentrations observed during clinical use. Nevertheless, we have previously demonstrated that, even at 10 μM, lidocaine showed a tendency to augment the relaxation produced by cAMP-elevating agents (Nakahara et al., 2000). Therefore, we consider that lidocaine at a therapeutic concentration range might potentiate the relaxant effects of cAMP-elevating agents on muscarinic receptor-mediated airway smooth muscle contractions.

In summary, lidocaine potentiates the relaxant effect of agents that activate adenylyl cyclase in bovine tracheal smooth muscle preparations precontracted with methacholine. The potentiation seems to be due to the blockade by lidocaine of muscarinic M_2 receptor-mediated signal transduction. Thus, it is likely that lidocaine exerts a synergistic effect on the responses to agents that activate adenylyl cyclase in the bronchoconstriction associated with the enhanced parasympathetic nerve activity.

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