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Effects of Lidocaine on Intracellular Ca^{2+} and Tension in Airway Smooth Muscle

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Background: **Many studies have demonstrated that lidocaine directly relaxes airway smooth muscle. The underlying mechanisms, especially in relation to Ca^{2+} mobilization, remain to be elucidated.**

Methods: **Using front-surface fluorometry and fura-2-loaded porcine tracheal smooth muscle strips, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and isometric tension were simultaneously measured.**

Results: **In cases of 40 mM K^+-induced contraction and 1 μM acetylcholine (ACh)-induced contraction, the cumulative application of lidocaine (10^{-6}~3×10^{-3} M) caused a concentration-dependent decrease in $[Ca^{2+}]_i$ and tension, and almost complete relaxation. To examine the effect of lidocaine on Ca^{2+} sensitivity of the contractile apparatus, the $[Ca^{2+}]_i$-tension relationship was determined by changing the extracellular Ca^{2+} concentration during 40 mM K^+ induced depolarization, with and without treatment with lidocaine. Although treatment with 1 mM lidocaine inhibited increases in both $[Ca^{2+}]_i$ and tension induced by extracellular Ca^{2+}, it had little effect on the $[Ca^{2+}]_i$–tension relationship. In the presence of 1 μM ACh, the $[Ca^{2+}]_i$–tension relationship shifted markedly to the left, thereby indicating an increase in Ca^{2+} sensitivity of the contractile apparatus; this shift was inhibited by 1 mM lidocaine. In the absence of extracellular Ca^{2+}, 1 mM lidocaine inhibited the release of stored Ca^{2+} induced by 1 μM ACh, but not that by 20 mM caffeine.**

Conclusions: **Lidocaine directly relaxes airway smooth muscle by decreasing $[Ca^{2+}]_i$. In addition, lidocaine inhibits the ACh-induced increase of Ca^{2+} sensitivity of the contractile apparatus, although it has little effect on Ca^{2+} sensitivity during high K^+ depolarization. The decrease in $[Ca^{2+}]_i$ is attributed to inhibition of the influx of extracellular Ca^{2+}, as induced by high K^+ depolarization and by ACh, and to the inhibition of the ACh-induced release of stored Ca^{2+}. (Key words: Anesthetics, local: lidocaine. Ions, calcium: calcium sensitivity; intracellular. Muscle, smooth: airway.)**

LIDOCAINE is often administered intravenously to suppress airway reflexes associated with tracheal intubation or tracheal suction. In addition, intravenous lidocaine has spasmolytic effects in the case of bronchospasm.[1] The mechanisms of this airway relaxant effect could include the following: interruption of reflex arcs;[2,3] inhibition of chemical mediator release;[4] and direct relaxant effect on airway smooth muscle.[5–7] In addition to neuronal effects, many studies demonstrated that lidocaine directly relaxes airway smooth muscle,[5–7] vascular smooth muscle,[8] and other smooth muscles. The mechanism of direct relaxant effects on smooth muscle of lidocaine may be caused by an effect on Ca^{2+} mobilization;[5,9,10] however, there has been no direct evidence to support this hypothesis with regard to airway as well as other smooth muscles.

Although it is generally accepted that the contraction of smooth muscle is primarily regulated by $[Ca^{2+}]_i$, smooth muscle contractile force does not simply depend on $[Ca^{2+}]_i$. Morgan and Morgan[11] showed, for the first time, that agonists can increase the effectiveness

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of intracellular Ca^{2+} on the contractile apparatus. More recently, it was shown that, in α-toxin or β-escin permeabilized smooth muscles, some agonists induce an enhanced sensitivity of myofilament to Ca^{2+}, through a G protein-mediated pathway.[12,13] In addition, it was clearly shown that contraction in intact smooth muscle strips can occur without any change in $[Ca^{2+}]_i$.[14] Thus, to clarify the mechanism underlying smooth muscle relaxation, it is important to know the change in $[Ca^{2+}]_i$. Therefore, we investigated the mechanism of lidocaine's direct relaxant effect on airway smooth muscle by simultaneously measuring tension and $[Ca^{2+}]_i$, using front-surface fluorometry and the Ca^{2+} indicator dye fura-2. The hypotheses to be tested in this study are whether: 1) lidocaine reduces $[Ca^{2+}]_i$, and 2) lidocaine changes the sensitivity of the contractile apparatus to $[Ca^{2+}]_i$.

Materials and Methods

Tissue Preparation

Tracheas were dissected from adult pigs at a local slaughterhouse using a protocol approved by the Animal Research Committee of Research Institute of Angiocardiology, Faculty of Medicine, Kyushu University. The tracheas were placed in preaerated ice-cold physiologic saline solution (PSS) and brought to our laboratory within 30 min of dissection. The lower end of the trachea, just above the first bronchus branching, three tracheal rings in length, was used for experiments. The posterior portion of the trachea was excised longitudinally, and the cartilages were detached. The mucosa and adventitial tissue were carefully removed under microscopic observation. The muscle sheets were transversely cut into rectangular strips approximately 3 mm in length and 1 mm in width. All tissue preparations in the laboratory were performed in aerated PSS.

Fura-2 Loading

Tracheal strips were loaded with the Ca^{2+} indicator dye, fura-2, in the form of acetoxymethyl ester (fura-2/AM). The strips were incubated in 2 ml aerated (95% O_2: 5% CO_2) Dulbecco-modified Eagle's medium containing 50 μM fura-2/AM dissolved in dimethyl sulphoxide and 5% fetal bovine serum for 3 h at 37° C. After loading with fura-2, the strips were washed with normal PSS to remove dye in the extracellular space, and then were further incubated in normal PSS for at least 1 h to facilitate the deesterification of intracellular fura-2/AM and to equilibrate the strips before starting the measurements.

Measurement of Tension Development

Each strip was mounted vertically in a 6-ml quartz organ bath, which was maintained at 37° C and bubbled with 95% O_2 and 5% CO_2. The lower end of the strip was fixed by a small clip and the upper end of the strip was attached by a small clip and thread to a force transducer (TB-612T, Nihon Koden, Japan) for recording of isometric tension. During the 1-h fura-2 equilibration period, the strips were stimulated with 40 mM K^+ at 5–10-min intervals, and muscle length was increased stepwise after each stimulation until the developed tension reached a maximum. When exposed to 40 mM K^+, most strips produced stable tension within 5–10 min, after forming a transient peak tension or not. Strips that showed an instability in tension, as induced by 40 mM K^+, were excluded from the study.

The responsiveness of each strip to 40 mM K^+ was recorded before starting the experimental protocol, because almost maximum, reproducible responses of tension to high K^+ depolarization were obtained at this concentration of K^+. The developed tension was expressed as a percentage, assuming the values in normal (5.9 mM K^+) PSS and steady state of 40 mM K^+ PSS to be 0% and 100%, respectively.

Measurement of Fura-2 Fluorescence

Changes in fluorescence intensity of the fura-2-Ca^{2+} complex were monitored using a front-surface fura-2 fluorometer (model CAM-OF1), specifically designed in collaboration with Japan Spectroscopic (Tokyo, Japan). Details of our front-surface fluorometry system have been described elsewhere.[14–16] In brief, two wavelengths of excitation light (340 and 380 nm) were obtained spectroscopically from a Xenon light source. Strips were illuminated by guiding the two alternating (400 Hz) wavelengths of excitation light through quartz optic fibers. Surface fluorescence of the strip was collected by glass optic fibers and introduced through a 500-nm band pass filter into a photomultiplier. Thus, we measured the fura-2 fluorescence intensity of 500-nm emission light, which was induced by alternating two wavelengths of excitation light (340 and 380 nm).

The ratio of the fluorescence intensities (fluorescence ratio) at 340 nm excitation (F_{340}) to that at 380 nm excitation (F_{380}) was monitored to estimate changes in $[Ca^{2+}]_i$ and expressed as a percentage, assuming the val-

ues in normal PSS (5.9 mM K^+) and steady state of 40 mM K^+ PSS to be 0% and 100%, respectively. The absolute value of $[Ca^{2+}]_i$ was determined using the equation of Grynkiewicz *et al.*[17] The minimum and maximum fluorescence ratios were determined by the addition of 25 μM ionomycin to Ca^{2+}-free PSS containing 2 mM EGTA, followed by replacement with normal PSS (1.25 mM Ca^{2+}), respectively. Using this equation, the calculated $[Ca^{2+}]_i$ in normal PSS (0%) and steady state of 40 mM K^+ PSS (100%) was 90 ± 14 and 499 ± 54 nM (n = 8), respectively.

Representative simultaneous recording of changes in fluorescence and tension induced by 40 mM K^+ depolarization is shown in figure 1.

Experimental Protocols

To examine the effect of lidocaine on Ca^{2+} influx induced by high K^+ depolarization or by acetylcholine (ACh), lidocaine (10^{-6}~3×10^{-3} M) was cumulatively applied on the steady state of elevations of $[Ca^{2+}]_i$ and tension induced by either 40 mM K^+ or 1 μM ACh.

To examine the effect of lidocaine on Ca^{2+} sensitivity of the contractile apparatus during contractions induced by 40 mM K^+ depolarization, we determined the $[Ca^{2+}]_i$–tension relationship by changing the extracellular Ca^{2+} concentration, as follows. After 10 min incubation in Ca^{2+}-free PSS containing 2 mM EGTA, and then 5 min incubation in Ca^{2+}-free PSS without EGTA, strips were immersed in Ca^{2+}-free 40 mM K^+ solution. Next, the extracellular Ca^{2+} concentration was stepwise increased by the cumulative addition of $CaCl_2$. To examine the effect of lidocaine, 1 mM lidocaine was applied at the time of replacement with Ca^{2+}-free PSS without EGTA. To determine the effect of lidocaine on Ca^{2+} sensitivity of the contractile apparatus during contractions induced by ACh, 1 μM ACh was applied after 5 min incubation in Ca^{2+}-free PSS containing 2 mM EGTA. The protocols used after that were similar to the case of contractions induced by 40 mM K^+ depolarization, excepting the existence of ACh.

Effects of lidocaine on the Ca^{2+} release by caffeine or ACh was determined as follows. After 10 min incubation in Ca^{2+}-free PSS containing 2 mM EGTA, 20 mM caffeine or 1 μM ACh was applied twice at 10-min intervals. The time of application of caffeine and ACh was 2 min and 5 min, respectively. Lidocaine (1 mM) was applied 5 min before and during the first application of caffeine or ACh. Thus, during the second application of caffeine or ACh, lidocaine was absent.

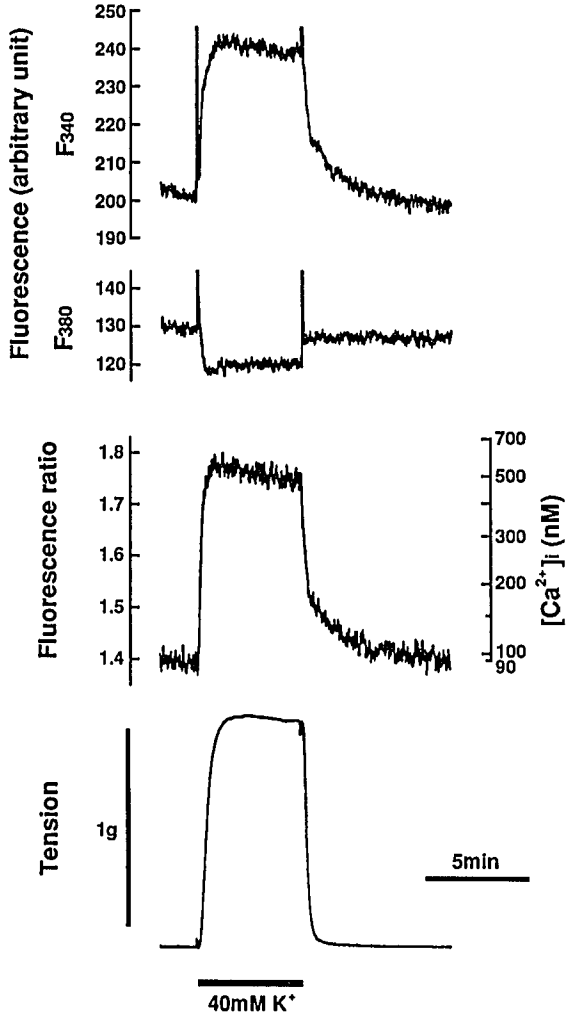

Fig. 1. Representative time course of fluorescence and tension induced by 40 mM K^+ depolarization. The first and second traces from the top show changes in 500-nm fluorescence intensities obtained at 340 nm (F_{340}) and 380 nm (F_{380}) excitations, respectively. The third trace shows changes in fluorescence ratio of F_{340} to F_{380}. The lowest trace shows tension development.

Solutions and Drugs

Normal PSS was of the following composition (in mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.25, and D-glucose 11.5. High K^+ PSS was identical to normal PSS, except for an equimolar substitution of KCl for NaCl. The Ca^{2+}-free version of PSS was produced by exclusion of $CaCl_2$ from the composition of normal PSS. Physiologic saline solution was bubbled with 95% O_2 and 5% CO_2, with a resulting pH of 7.4 at 37° C. Fura-2/AM and EGTA [ethylene glycol-bis(β-aminoethyl ether)N,N,N′,N′-tetra-

acetic acid] were purchased from Dojindo (Kumamoto, Japan). Lidocaine hydrochloride was from Fujisawa Pharmaceutical (Osaka, Japan). Acetylcholine chloride was from Sigma Chemical (St. Louis, MO). Caffeine was from Katayama Chemical (Osaka, Japan).

Data Analysis

The measured values were expressed as mean ± SE (n = number of observations). For each observation, a strip from a different animal was used. One-way analysis of variance for repeated measurements was used to determine the concentration-dependent effects. Two-way analysis of variance for repeated measurements was used to determine the statistical significance of lidocaine's effect on the contractions induced by extracellular Ca^{2+} during high K^+ depolarization. Analysis of covariance was used to determine the statistical significance of the shift of the $[Ca^{2+}]_i$–tension relationship. For the rest of the measurements, unpaired Student's *t* test was used. *P* values of less than 0.05 were considered to be significant. The IC_{50} values (the lidocaine concentration that decreases $[Ca^{2+}]_i$ and tension to 50% of the maximal response) were calculated, using the four-parameter logistic equation reported by De Lean *et al.*[18]

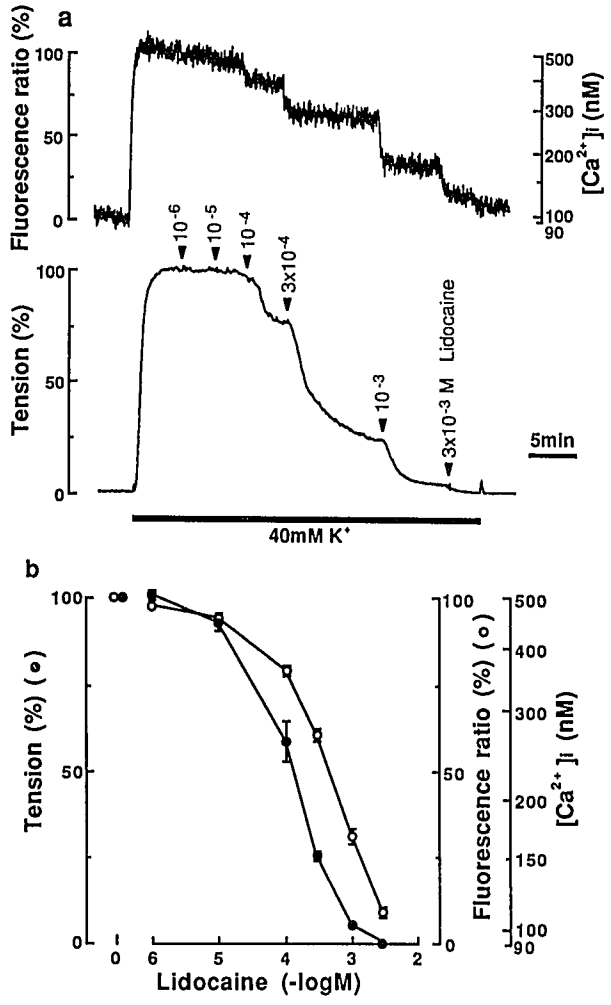

Fig. 2. Effect of lidocaine on elevated $[Ca^{2+}]_i$ and tension induced by high K^+ depolarization. (*A*) Representative time course of the effect of cumulatively applied lidocaine (10^{-6}~3×10^{-3} M) on elevated $[Ca^{2+}]_i$ and tension induced by 40 mM K^+ depolarization. (*B*) Concentration-dependent effect of lidocaine on elevated $[Ca^{2+}]_i$ (○) and tension (●) induced by 40 mM K^+ depolarization. Lidocaine was cumulatively applied to the steady state of 40 mM K^+-induced $[Ca^{2+}]_i$ elevation and contraction. Plots are means of five preparations, with SE shown by vertical bars.

Results

Effect of Lidocaine on Increased $[Ca^{2+}]_i$ and Tension Induced by High K^+ Depolarization

When lidocaine was cumulatively applied (10^{-6}~3×10^{-3} M) to the steady state of 40 mM K^+-induced $[Ca^{2+}]_i$ elevation and contraction, a concentration-dependent reduction in $[Ca^{2+}]_i$ and tension occurred (fig. 2). The application of 3×10^{-3} M lidocaine reduced $[Ca^{2+}]_i$ and tension to 9.2 ± 1.7% (110 nM) and 0.4 ± 0.2%, respectively (n = 5). The IC_{50} values (concentrations of lidocaine which induced 50% of the changes obtained with 3×10^{-3} M lidocaine) for $[Ca^{2+}]_i$ and tension were approximately 3.4×10^{-4} M and 1.2×10^{-4} M, respectively. When lidocaine was cumulatively applied during the resting state in normal PSS, no significant change in $[Ca^{2+}]_i$ and tension was detected (data not shown). After washing out lidocaine with normal PSS for 10 min, 40 mM K^+ caused the same extent of response, thereby indicating that the effect of lidocaine was reversible.

Effect of Lidocaine on Increased $[Ca^{2+}]_i$ and Tension Induced by ACh

Figure 3A shows a representative time course of the effect of cumulative application of lidocaine (10^{-6}~3×10^{-3} M) on elevated $[Ca^{2+}]_i$ and tension induced by 1 μM ACh. ACh induced rapid rises in $[Ca^{2+}]_i$ and tension with gradual decreases to a steady level within 10 min. These steady levels of $[Ca^{2+}]_i$ and tension were maintained during a 1-h observation period. Cumulative application of lidocaine, at the steady level, caused a

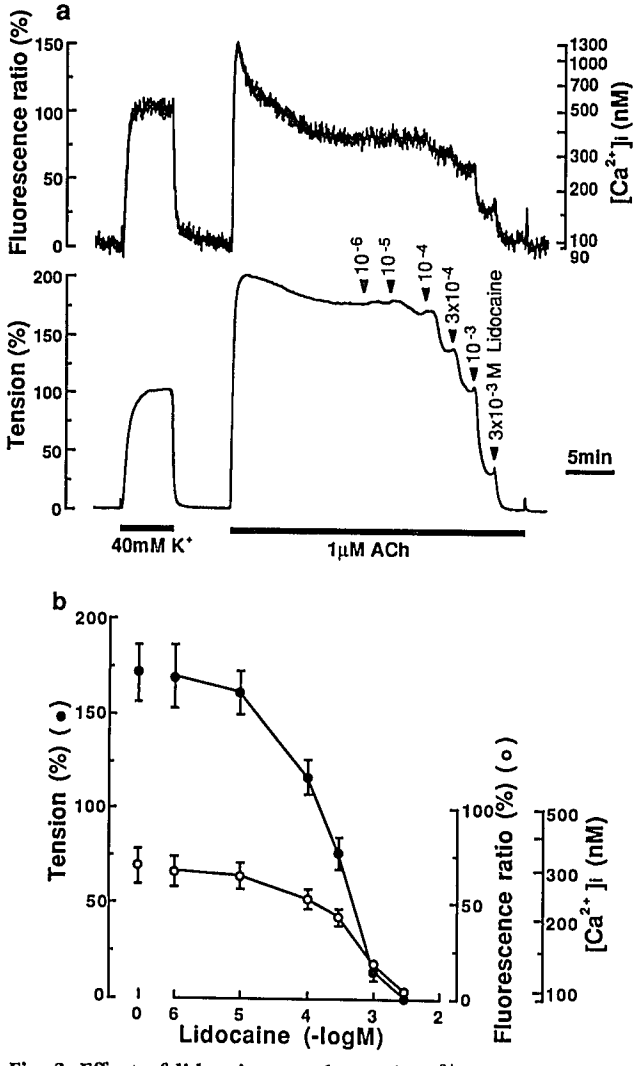

Fig. 3. Effect of lidocaine on elevated $[Ca^{2+}]_i$ and tension induced by 1 μM ACh. (*A*) Representative time course of the effect of cumulatively applied lidocaine (10^{-6}~3×10^{-3} M) on elevated $[Ca^{2+}]_i$ and tension induced by 1 μM ACh. (*B*) Concentration-dependent effect of lidocaine on elevated $[Ca^{2+}]_i$ (○) and tension (●) induced by 1 μM ACh. Lidocaine was cumulatively applied to the steady state of 1 μM ACh-induced $[Ca^{2+}]_i$ elevation and contraction. Percentages of fluorescence ratio and tension were obtained by taking values for normal (5.9 mM K^+) and 40 mM K^+ PSS to be 0 and 100%, respectively. Plots are means of five preparations, with SE shown by vertical bars.

concentration-dependent reduction in $[Ca^{2+}]_i$ and tension (fig. 3B). The application of 3×10^{-3} M lidocaine reduced $[Ca^{2+}]_i$ and tension to 4.0 ± 1.0% (99 nM) and 0.6 ± 0.4%, respectively (n = 5). The IC_{50} values (concentrations of lidocaine which induced 50% of the changes obtained with 3×10^{-3} M lidocaine) for $[Ca^{2+}]_i$ and tension were approximately 2.8×10^{-4} M and 1.5×10^{-4} M, respectively.

Effect of Lidocaine on $[Ca^{2+}]_i$ and Tension Development Induced by Changes in Extracellular Ca^{2+} Concentration during High K^+ Depolarization

Figure 4A shows a representative time course of changes in $[Ca^{2+}]_i$ and tension induced by the cumulative application of $CaCl_2$ in Ca^{2+}-free 40 mM K^+ solution. In response to the stepwise increment of extracellular Ca^{2+} concentration (0.0125–2.5 mM), $[Ca^{2+}]_i$ and tension increased in a concentration-dependent manner. $[Ca^{2+}]_i$ increased from −26.1 ± 2.0% (43 nM) at 0 mM extracellular Ca^{2+} to 108.9 ± 6.1% (579 nM) at 2.5 mM extracellular Ca^{2+}, and tension increased from 0 ± 0% to 83.1 ± 11.2% (n = 10). Treatment with 1 mM lidocaine 8 min before and during cumulative application of extracellular Ca^{2+} significantly inhibited increases in $[Ca^{2+}]_i$ and tension ($P < 0.01$ for both, by two-way analysis of variance) (fig. 4B, C). In the lidocaine-treated strips, with the stepwise increment of extracellular Ca^{2+} concentration (0.0125–5 mM), $[Ca^{2+}]_i$ increased from −15.8 ± 1.4% (60 nM) at 0 mM extracellular Ca^{2+} to 73.4 ± 6.3% (328 nM) at 5 mM extracellular Ca^{2+}, and tension increased from 0 ± 0% to 33.2 ± 13.2% (n = 5).

Effect of Lidocaine on $[Ca^{2+}]_i$ and Tension Development Induced by Changes in Extracellular Ca^{2+} Concentration during High K^+ Depolarization in the Presence of ACh

Figure 5A shows a representative time course of changes in $[Ca^{2+}]_i$ and tension induced by the cumulative application of $CaCl_2$ during depolarization with 40 mM K^+ in the presence of 1 μM ACh. In response to the stepwise increment of extracellular Ca^{2+} concentration (0.0125–2.5 mM), $[Ca^{2+}]_i$ and tension increased in a concentration-dependent manner. In the presence of ACh, the increase in $[Ca^{2+}]_i$ was similar to that in the absence of ACh; however, the increase in tension was much greater than that in the absence of ACh (figs. 4A and 5A). $[Ca^{2+}]_i$ increased from −21.0 ± 3.4% (51 nM) at 0 mM extracellular Ca^{2+} to 111.8 ± 9.5% (608 nM) at 2.5 mM extracellular Ca^{2+}, and tension increased from 1.6 ± 0.9% to 185.0 ± 13.4% (n = 5). Treatment with 1 mM lidocaine 8 min before and during cumulative application of extracellular Ca^{2+} significantly inhibited increases in $[Ca^{2+}]_i$ and tension ($P < 0.01$ for both, by two-way analysis of variance) (fig. 5B, C). In

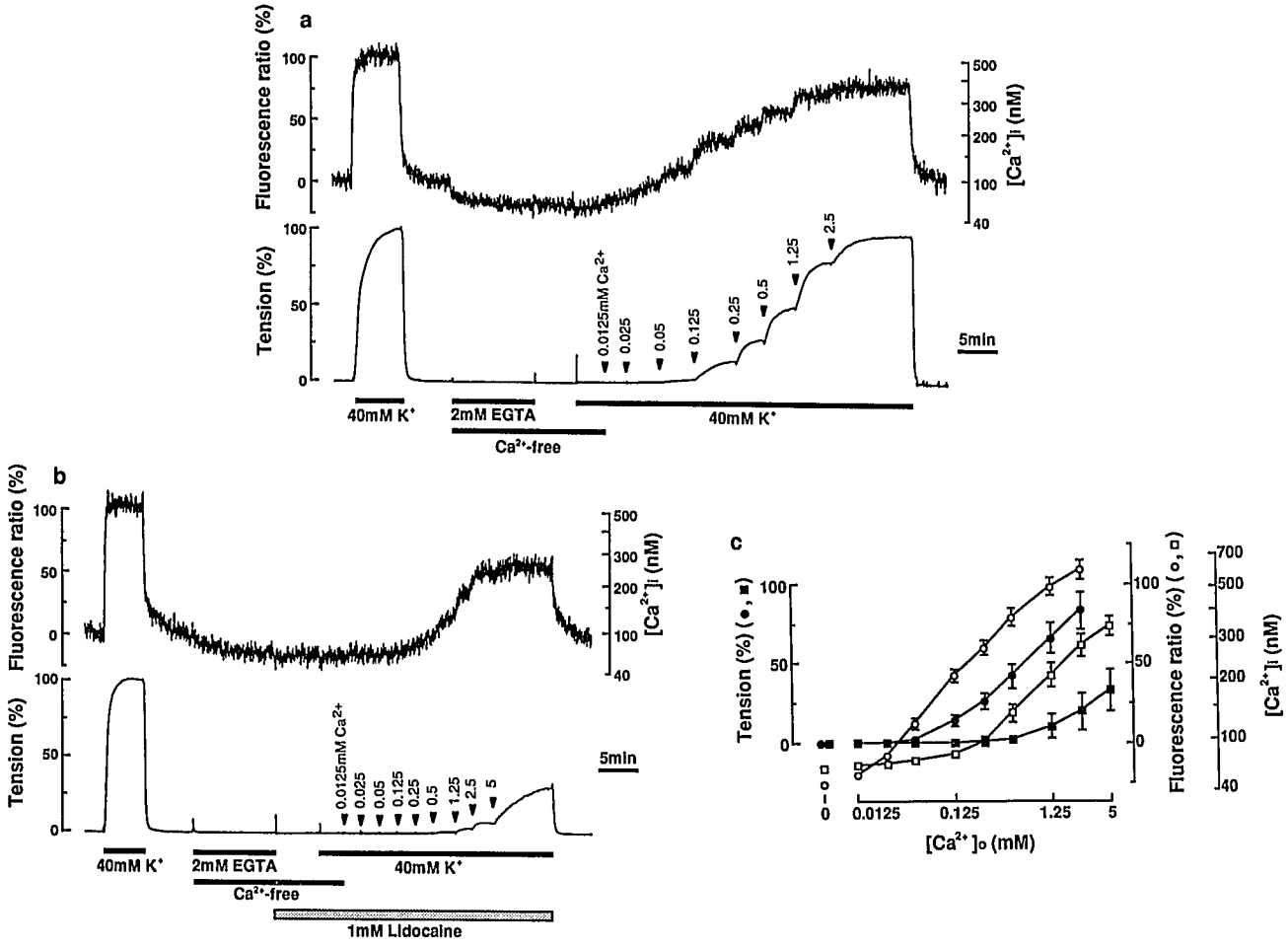

Fig. 4. Effect of 1 mM lidocaine on $[Ca^{2+}]_i$ and tension development induced by cumulative application of external Ca^{2+} (0.0125–2.5 or 5 mM) during 40 mM K^+ depolarization. (*A*) Representative time course of changes in $[Ca^{2+}]_i$ and tension induced by the cumulative application of $CaCl_2$ in Ca^{2+}-free 40 mM K^+ solution. (*B*) Representative time course of changes in $[Ca^{2+}]_i$ and tension with 1 mM lidocaine pretreatment. (*C*) Effect of 1 mM lidocaine on the increase in $[Ca^{2+}]_i$ (□) and tension development (■) induced by changes in extracellular Ca^{2+} during 40 mM K^+ depolarization. Controls: $[Ca^{2+}]_i$ (○) and tension (●) without lidocaine. Plots are means of ten (control) or five (with lidocaine) preparations, with SE shown by vertical bars.

the lidocaine-treated strips, with the stepwise increment of extracellular Ca^{2+} concentration (0.0125–5 mM), $[Ca^{2+}]_i$ increased from −19.4 ± 4.4% (54 nM) at 0 mM extracellular Ca^{2+} to 92.6 ± 6.7% (443 nM) at 5 mM extracellular Ca^{2+}, and tension increased from 0.8 ± 0.2% to 123.2 ± 11.3% (n = 5).

Effect of Lidocaine on $[Ca^{2+}]_i$-Tension Relationship

The $[Ca^{2+}]_i$-tension relationships in case of the extracellularly applied Ca^{2+}-induced contraction during high K^+ depolarization, in the presence or absence of ACh, with or without lidocaine-treatment, were evaluated from data in figures 4C and 5C (fig. 6). The $[Ca^{2+}]_i$-tension relationship in the presence of ACh shifted to the left of that in the absence of ACh. Treatment with 1 mM lidocaine inhibited this left side shift of the $[Ca^{2+}]_i$-tension relationship elicited by ACh. However, the $[Ca^{2+}]_i$-tension relationship in the absence of ACh was little affected by 1 mM lidocaine.

Effect of Lidocaine on $[Ca^{2+}]_i$ and Tension Development Induced by Caffeine in the Absence of Extracellular Ca^{2+}

Figure 7A shows a representative time course of $[Ca^{2+}]_i$ and tension development induced by repeated applications of 20 mM caffeine in Ca^{2+}-free solution containing 2 mM EGTA. The application of 20 mM caffeine

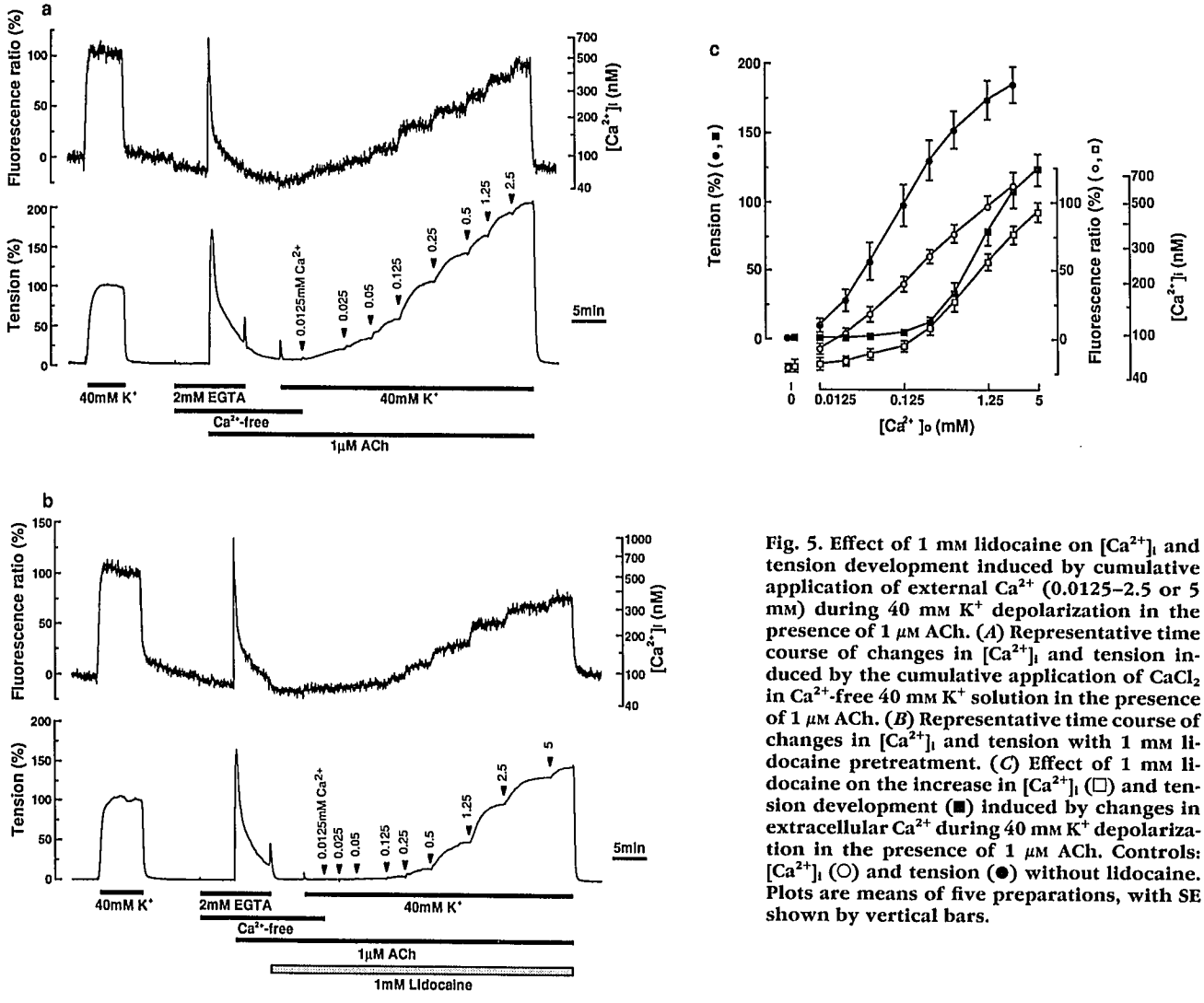

Fig. 5. Effect of 1 mM lidocaine on $[Ca^{2+}]_i$ and tension development induced by cumulative application of external Ca^{2+} (0.0125–2.5 or 5 mM) during 40 mM K^+ depolarization in the presence of 1 μM ACh. (*A*) Representative time course of changes in $[Ca^{2+}]_i$ and tension induced by the cumulative application of $CaCl_2$ in Ca^{2+}-free 40 mM K^+ solution in the presence of 1 μM ACh. (*B*) Representative time course of changes in $[Ca^{2+}]_i$ and tension with 1 mM lidocaine pretreatment. (*C*) Effect of 1 mM lidocaine on the increase in $[Ca^{2+}]_i$ (□) and tension development (■) induced by changes in extracellular Ca^{2+} during 40 mM K^+ depolarization in the presence of 1 μM ACh. Controls: $[Ca^{2+}]_i$ (○) and tension (●) without lidocaine. Plots are means of five preparations, with SE shown by vertical bars.

for 2 min in the absence of extracellular Ca^{2+} caused a transient increase in $[Ca^{2+}]_i$ and tension. With the repeated application of caffeine at 10-min intervals, the caffeine-induced increase in $[Ca^{2+}]_i$ and tension declined compared with findings with the first application. Treatment with 1 mM lidocaine 5 min before and during the first application of caffeine had no effect on increases of $[Ca^{2+}]_i$ and tension induced by caffeine, in either the first or the second applications (fig. 7B, C).

Effect of Lidocaine on $[Ca^{2+}]_i$ and Tension Development Induced by ACh in the Absence of Extracellular Ca^{2+}

Figure 8A shows a representative time course of $[Ca^{2+}]_i$ and tension development induced by repeated applications of 1 μM ACh in Ca^{2+}-free solution containing 2 mM EGTA. The application of 1 μM ACh for 5 min in the absence of extracellular Ca^{2+} caused a transient increase in $[Ca^{2+}]_i$ and tension. With the repeated application of ACh at 10-min intervals, the ACh-induced increase in $[Ca^{2+}]_i$ and tension greatly declined compared with findings with the first application. Treatment with 1 mM lidocaine 5 min before and during the first application of ACh significantly inhibited the increases of $[Ca^{2+}]_i$ and tension induced by the first application of ACh. With the second application of ACh, after washing out of lidocaine, increases in $[Ca^{2+}]_i$ and tension were significantly greater than those of the second application of control (fig. 8B, C). In addition, the developed tension, as induced by the second application

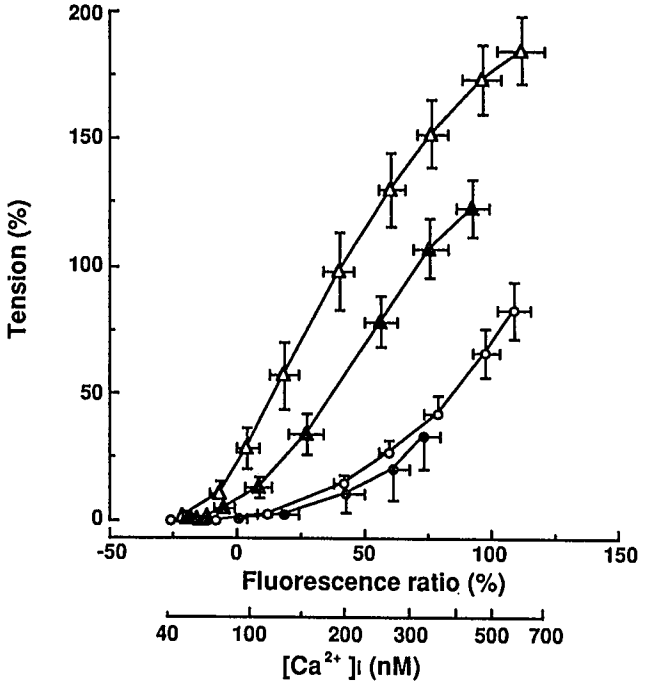

Fig. 6. Effect of lidocaine on the $[Ca^{2+}]_i$-tension relationship. The $[Ca^{2+}]_i$-tension relationship was obtained from data in figures 4 and 5. ○ and ● indicate values obtained from contractions induced by changes in extracellular Ca^{2+} during 40 mM K^+ depolarization, with (●) or without (○) treatment with 1 mM lidocaine. △ and ▲ indicate values obtained from contractions induced by changes in extracellular Ca^{2+} during 40 mM K^+ depolarization in the presence of 1 μM ACh, with (▲) or without (△) treatment with 1 mM lidocaine.

of ACh after washing out of lidocaine, was greater than that seen with the first application, although the peak $[Ca^{2+}]_i$ elevation was less.

Discussion

Based on the current understanding of excitation–contraction coupling in airway smooth muscle, the following two mechanisms play major roles; those dependent on changes in the surface membrane potential (electromechanical coupling) and those independent of the surface membrane potential (pharmacomechanical coupling).[19] Electromechanical coupling includes G protein-mediated activation of channels effecting surface membrane depolarization, activation of voltage-dependent Ca^{2+} channels, and influx of Ca^{2+} into the cell. Pharmacomechanical coupling is driven by G protein-mediated activation of phospholipase C (PLC), which catalyzes hydrolysis of membrane phosphatidylinositides, forming the second messengers, inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 releases Ca^{2+} from sarcoplasmic reticulum. Through these two pathways, the increase in $[Ca^{2+}]_i$ occurs. Ca^{2+} binds to calmodulin to activate myosin light chain kinase that catalyzes phosphorylation of the myosin light chain, activation of actomyosin ATPase, and contraction.[20] Thus, $[Ca^{2+}]_i$ primarily regulates smooth muscle contraction through electromechanical and pharmacomechanical couplings. However, smooth muscle contractile force does not simply depend on $[Ca^{2+}]_i$. Some agonists cause an increase in the sensitivity of myofilament to $[Ca^{2+}]_i$. This mechanism is also included in the pharmacomechanical coupling.[21] To clarify mechanisms for lidocaine-induced relaxation of airway smooth muscle, we investigated the effect of lidocaine on these mechanisms; Ca^{2+} influx through voltage-dependent Ca^{2+} channel, Ca^{2+} release, and change in Ca^{2+} sensitivity of myofilament.

Inhibition of Ca^{2+} Influx by Lidocaine

In cases of high K^+ depolarization and ACh-induced contractions, lidocaine (10^{-6}~3×10^{-3} M) caused a concentration-dependent decrease in $[Ca^{2+}]_i$ and tension. Treatment with 1 mM lidocaine inhibited the extracellularly applied Ca^{2+}-induced increases in $[Ca^{2+}]_i$ and tension during high K^+ depolarization, in the presence or absence of ACh. Cumulative application of lidocaine (10^{-6}~3×10^{-3} M) during the resting state led to no significant change in $[Ca^{2+}]_i$ and tension. These observations suggest that lidocaine inhibits the influx of extracellular Ca^{2+} induced by high K^+ depolarization and by ACh and, thus, decreases $[Ca^{2+}]_i$ to cause relaxation. In addition, the lidocaine-induced inhibition in $[Ca^{2+}]_i$ and tension during high K^+ depolarization is probably not related to inhibition of the effect of ACh, which is assumed to be released from nerve endings by high K^+ depolarization, because Weiss *et al.*[5] reported that pretreatment with 7 μM atropine did not modify the lidocaine-induced inhibition of hypertonic potassium contractions.

Consistent with the idea that lidocaine inhibits the influx of extracellular Ca^{2+}, Hay and Wadsworth[22] stated that the inhibitory action of lidocaine on KCl responses in rat vas deferens was reversed by raising the extracellular Ca^{2+} concentration. Spedding and Berg[23] reported that drugs acting on Na^+ channels, such as local anesthetics including lidocaine, could also interact with Ca^{2+} channels, as deduced from similar observations in guinea-pig taenia. More direct evidence for the inhibition of Ca^{2+} influx by lidocaine in elec-

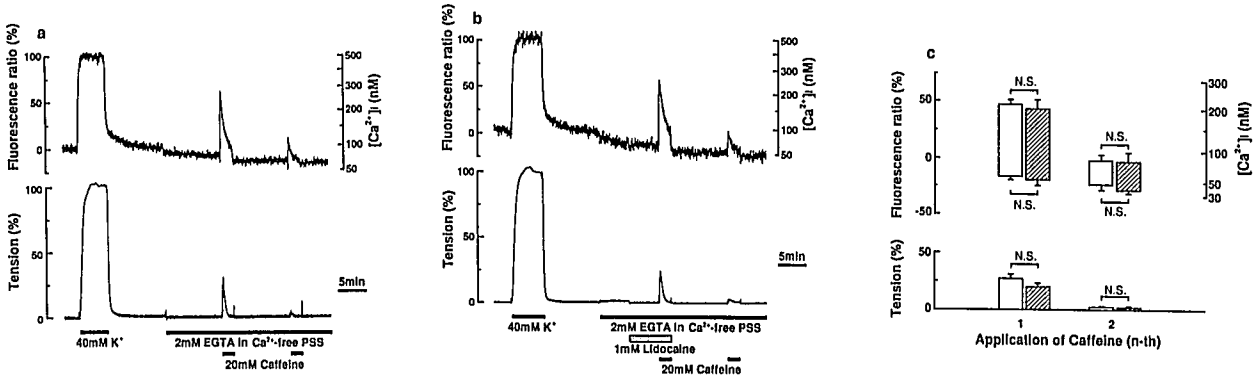

Fig. 7. Effect of 1 mM lidocaine on $[Ca^{2+}]_i$ and tension development induced by 20 mM caffeine in the absence of extracellular Ca^{2+}. (*A*) Representative time course of $[Ca^{2+}]_i$ and tension development induced by repeated applications of 20 mM caffeine in Ca^{2+}-free solution containing 2 mM EGTA. (*B*) Representative time course of $[Ca^{2+}]_i$ and tension development induced by repeated applications of 20 mM caffeine in Ca^{2+}-free solution containing 2 mM EGTA with the treatment with 1 mM lidocaine before and during the first application of caffeine. Lidocaine was washed out at the end of the first application of caffeine. (*C*) $[Ca^{2+}]_i$ and tension development in response to repeated applications of caffeine, without (open columns) and with (hatched columns) 1 mM lidocaine treatment on the first application of caffeine. The bottom and the top of each column indicate the $[Ca^{2+}]_i$ and tension just before and at the peak levels obtained at the nth application of caffeine, respectively. The vertical bars at the bottom and the top of each column show SE (n = 4).

trophysiologic experiments was obtained using non-smooth muscle cells. Josephson[24] reported that lidocaine decreased the Ca current in embryonic chick ventricular myocytes, in a concentration-dependent manner (10–1,000 μM). Also, Elliott[25] reported that, in rat sympathetic nerves, lidocaine blocked Ca-spikes with an IC_{50} of 0.77 mM, despite a less potent effect compared with the action on Na-spikes with an IC_{50} of 163 μM. The type of Ca^{2+} channel directly affected by lidocaine was not clarified in these studies. Questions of whether lidocaine inhibits the Ca^{2+} channel and which type of channel is involved in this inhibition in airway smooth muscle remain to be elucidated.

Effects of Lidocaine on Ca^{2+} Release

To examine the effect of lidocaine on Ca^{2+} release, two distinct stimulants, caffeine and ACh, were used. Caffeine causes Ca^{2+} release by facilitating the Ca^{2+}-

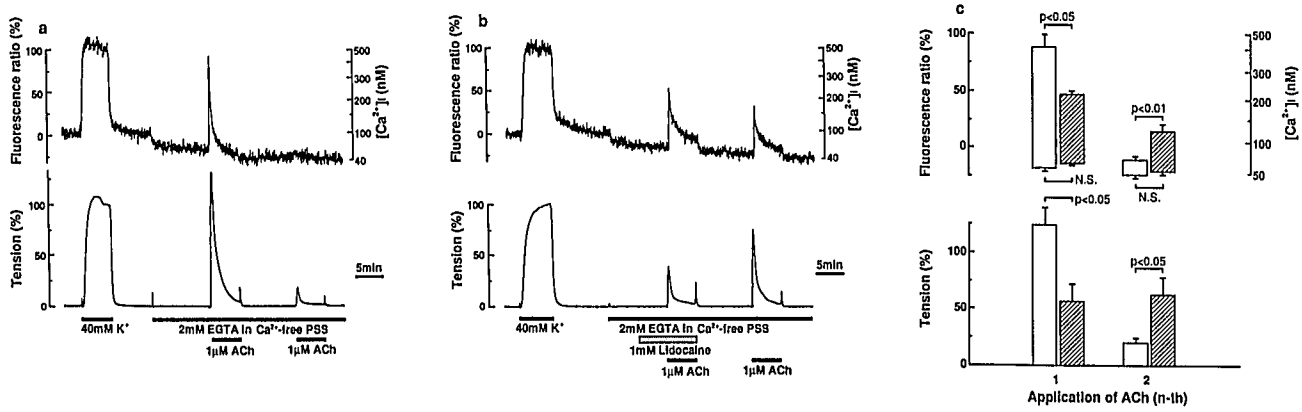

Fig. 8. Effect of 1 mM lidocaine on $[Ca^{2+}]_i$ and tension development induced by 1 μM ACh in the absence of extracellular Ca^{2+}. (*A*) Representative time course of $[Ca^{2+}]_i$ and tension development induced by repeated applications of 1 μM ACh in Ca^{2+}-free solution containing 2 mM EGTA. (*B*) Representative time course of the effect of 1 mM lidocaine on $[Ca^{2+}]_i$ and tension development induced by 1 μM ACh in Ca^{2+}-free solution containing 2 mM EGTA. Lidocaine was administered 5 min before the first application of ACh. After the first application of ACh for 5 min, lidocaine was washed out with Ca^{2+}-free solution for 10 min, followed by the second application of ACh. (*C*) $[Ca^{2+}]_i$ and tension development in response to repeated applications of ACh, without (open columns) and with (hatched columns) 1 mM lidocaine treatment on the first application of ACh. The bottom and the top of each column indicate the $[Ca^{2+}]_i$ and tension just before and at the peak levels obtained at the nth application of ACh, respectively. The vertical bars at the bottom and the top of each column show SE (n = 4).

induced Ca^{2+} release mechanism, while ACh causes Ca^{2+} release by pharmacomechanical coupling mediated by IP_3.[26]

In the absence of extracellular Ca^{2+}, 1 mM lidocaine inhibited the increases in $[Ca^{2+}]_i$ and tension induced by ACh, but not by caffeine. This observation indicates that lidocaine inhibits the ACh-induced release of intracellularly stored Ca^{2+}, without affecting the Ca^{2+}-induced Ca^{2+} release mechanism. Another consideration is that lidocaine depletes the ACh sensitive Ca^{2+} store, but this possibility can be excluded because the second application of ACh after washing out of lidocaine induced a greater $[Ca^{2+}]_i$ elevation than did the second application of ACh in the control experiment (fig. 8).

Effects of Lidocaine on Myofilament Ca^{2+} Sensitivity

The $[Ca^{2+}]_i$-tension relationship in the presence of ACh shifted markedly to the left of that in the absence of ACh. In other words, in the presence of ACh, given $[Ca^{2+}]_i$ causes a greater tension than in the absence of ACh, indicating that the Ca^{2+} sensitivity of the contractile apparatus is increased by ACh. A similar finding, that muscarinic agonists increase myofilament Ca^{2+} sensitivity in airway smooth muscle, has been reported by others.[27–29] Lidocaine (1 mM) inhibited the left side shift of the $[Ca^{2+}]_i$-tension relationship elicited by ACh, but had little effect on the $[Ca^{2+}]_i$-tension relationship in the absence of ACh. This observation suggests that 1 mM lidocaine strongly inhibits the increase in Ca^{2+} sensitivity of the contractile apparatus elicited by ACh, but has little effect on the "native" $[Ca^{2+}]_i$-tension relationship during the Ca^{2+}-induced contraction. Tanaka and Hidaka[30] found that local anesthetics inhibit Ca^{2+}–calmodulin-dependent enzyme activities. They reported that lidocaine inhibited myosin light chain kinase (MLCK) from chicken gizzard with an IC_{50} of 6.8 mM. Nosaka *et al.*[31] also reported that 25 mM lidocaine inhibited the MLCK from turkey gizzard by 40%. Therefore, extremely high concentrations of lidocaine may inhibit the "native" Ca^{2+}-contraction pathway and cause a right side shift of the $[Ca^{2+}]_i$-tension relationship, but, in a concentration of 1 mM, the inhibitory effect of lidocaine on the "native" Ca^{2+}-contraction pathway is considered to be so weak that there is little effect on the $[Ca^{2+}]_i$-tension relationship, as observed in our study. However, the mechanism of the ACh-induced increase in Ca^{2+} sensitivity of the contractile apparatus is considered to be more easily inhibited by lidocaine in a concentration of 1 mM. A similar finding was reported that lidocaine ($\leq$1 mM) displaced to the right cumulative concentration-response curves to extracellular Ca^{2+} in 40 mM K^+-depolarized guinea-pig taenia, without depression of the maximal response.[23]

Possible Site of Action of Lidocaine

As already discussed, in pharmacomechanical coupling, DAG is produced together with IP_3 from membrane phosphatidylinositides with catalysis of PLC. 1,2-Diacylglycerol is known to activate protein kinase C (PKC), a putative candidate to induce an increase in Ca^{2+} sensitivity of the contractile apparatus.[32] Lidocaine may inhibit both ACh-induced IP_3 production and PKC activation, because, in the present study, lidocaine inhibited both the ACh-induced Ca^{2+} release and the ACh-induced increase in Ca^{2+} sensitivity of the contractile apparatus (figs. 6 and 8). Thus, it can be hypothesized that lidocaine may act on steps before the activation of PLC in the intracellular signal transduction system. The simplest explanation for this observation is that lidocaine is a ACh-receptor antagonist. However, lidocaine's inhibition of the ACh-induced contraction was described to be one of noncompetitive antagonism, because of a decrease in the ACh-induced maximal response and the nonparallel shift of the dose-response of ACh by lidocaine, and no modification by atropine on lidocaine's inhibition of hypertonic potassium contraction.[5] Thus, it is more likely that lidocaine may change affinity of the ACh receptor or act on the interaction of receptor-G protein or G protein-PLC. In any case, the site of action of lidocaine may be inside the plasma membrane.

If lidocaine does act inside the plasma membrane, the more membrane-soluble form of lidocaine would be more potent in relaxing smooth muscle. In support of this idea, Burdyga and Magura[33] found that 5 mM lidocaine caused a complete inhibition of the evoked action potentials and phasic contractions of the guinea pig ureter, and that this inhibitory action was significantly increased in high pH (pH 9), under which condition the lipid-soluble neutral form of lidocaine was increased. They also reported that the neutral local anesthetic benzocaine (1 mM) had a similar inhibitory effect, but the permanently charged local anesthetic QX-314 had no inhibitory effect. They assumed that the inhibitory action of the lipid-soluble neutral form of local anesthetics resulted from a blockade of the "slow" Na/Ca channels. A similar observation was reported by Spedding and Berg[23] that sodium salicylate,

which increases the negative surface charge of cell membranes, relaxed Ca^{2+}-induced contractions in K^+-depolarized guinea pig taenia pretreated with the cationic local anesthetics, including lidocaine, whereas salicylate did not affect the Ca^{2+}-induced contractions in smooth muscles pretreated with the neutral local anesthetic benzocaine. The observation that increasing the negative surface charge to facilitate the disposition of cationic local anesthetics in the membrane potentiated the inhibitory action on the Ca^{2+}-induced contraction during K^+ depolarization indicates that lidocaine may exert inhibitory effect on Ca^{2+} influx by its accumulation in cell membrane. Such an intramembranous location would be appropriate if lidocaine was to mediate effects on ACh-induced Ca^{2+} release and on ACh-induced increase of Ca^{2+} sensitivity of the contractile apparatus, because these processes are mediated by a G protein(s)-coupled mechanism involving hydrolysis of membrane lipids.

Clinical Relevance

The blood concentration of lidocaine clinically used in the prevention of ventricular arrhythmias is 2–5 μg/ml,[34] that is, around 10–20 μM. It is less than the concentrations of lidocaine that produced potent inhibitory effects on airway smooth muscle in the current study. However, these concentrations of lidocaine are likely to have a modest inhibitory effect on $[Ca^{2+}]_i$ and tension. In addition, we propose that, even with such a low concentration, lidocaine might have a modest effect in decreasing tension because of its inhibitory effect on myofilament Ca^{2+} sensitivity. On the other hand, Downes and Hirshman *et al.* demonstrated, in their *in vivo* studies, that intravenous or inhaled lidocaine blocks bronchoconstriction induced by the irritant reflex but not antigen-induced bronchoconstriction, and they presumed that the site of action is within the central nervous system.[2,3,35] In addition to this central effect, the current study indicates that the inhibitory effect of lidocaine on myofilament Ca^{2+} sensitivity, which is enhanced by various agonists, may contribute to the reduction of bronchoconstriction.

Lidocaine induces relaxation of tracheal smooth muscle by inducing a decrease in $[Ca^{2+}]_i$. In addition, lidocaine inhibits the ACh-induced increase of Ca^{2+} sensitivity of the contractile apparatus, although it has little effect on Ca^{2+} sensitivity during high K^+ depolarization. The decrease in $[Ca^{2+}]_i$ was attributed to inhibition of the influx of extracellular Ca^{2+}, as induced by high K^+ depolarization and by ACh, and to inhibition of the ACh-induced release of stored Ca^{2+}.

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