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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  $\mu$ M acetylcholine (ACh)-induced contraction, the cumulative application of lidocaine ( $10^{-6}$ ~ $3 \times 10^{-3}$  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  $\mu$ M 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  $\mu$ M 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.)

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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.<sup>1</sup> The mechanisms of this airway relaxant effect could include the following: interruption of reflex arcs;<sup>2,3</sup> inhibition of chemical mediator release;<sup>4</sup> and direct relaxant effect on airway smooth muscle.<sup>5-7</sup> In addition to neuronal effects, many studies demonstrated that lidocaine directly relaxes airway smooth muscle,<sup>5-7</sup> vascular smooth muscle,<sup>8</sup> 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,<sup>5,9,10</sup> 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<sup>11</sup> showed, for the first time, that agonists can increase the effectiveness

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

### 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  $\text{Ca}^{2+}$  indicator dye, fura-2, in the form of acetoxymethyl ester (fura-2/AM). The strips were incubated in 2 ml aerated (95%  $\text{O}_2$ : 5%  $\text{CO}_2$ ) Dulbecco-modified Eagle's medium containing 50  $\mu\text{M}$  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%  $\text{O}_2$  and 5%  $\text{CO}_2$ . 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  $\text{K}^+$  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  $\text{K}^+$ , 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  $\text{K}^+$ , were excluded from the study.

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

#### *Measurement of Fura-2 Fluorescence*

Changes in fluorescence intensity of the fura-2- $\text{Ca}^{2+}$  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.<sup>14–16</sup> 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  $[\text{Ca}^{2+}]_i$  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.*<sup>17</sup> The minimum and maximum fluorescence ratios were determined by the addition of 25  $\mu M$  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  $90 \pm 14$  and  $499 \pm 54$  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 ( $10^{-6}$ – $3 \times 10^{-3}$  M) was cumulatively applied on the steady state of elevations of  $[Ca^{2+}]_i$  and tension induced by either 40 mM  $K^+$  or 1  $\mu M$  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  $\mu M$  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  $\mu M$  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.

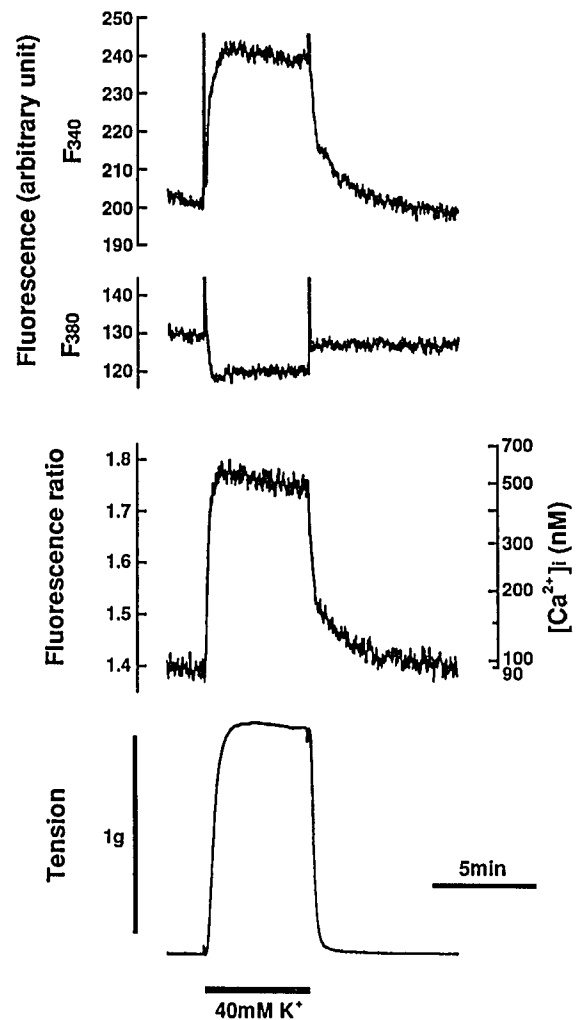


Fig. 1. Representative time course of fluorescence and tension induced by 40 mM  $K^+$  depolarization. The first and second traces from the top show changes in 500-nm fluorescence intensities obtained at 340 nm ( $F_{340}$ ) and 380 nm ( $F_{380}$ ) excitations, respectively. The third trace shows changes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

#### Solutions and Drugs

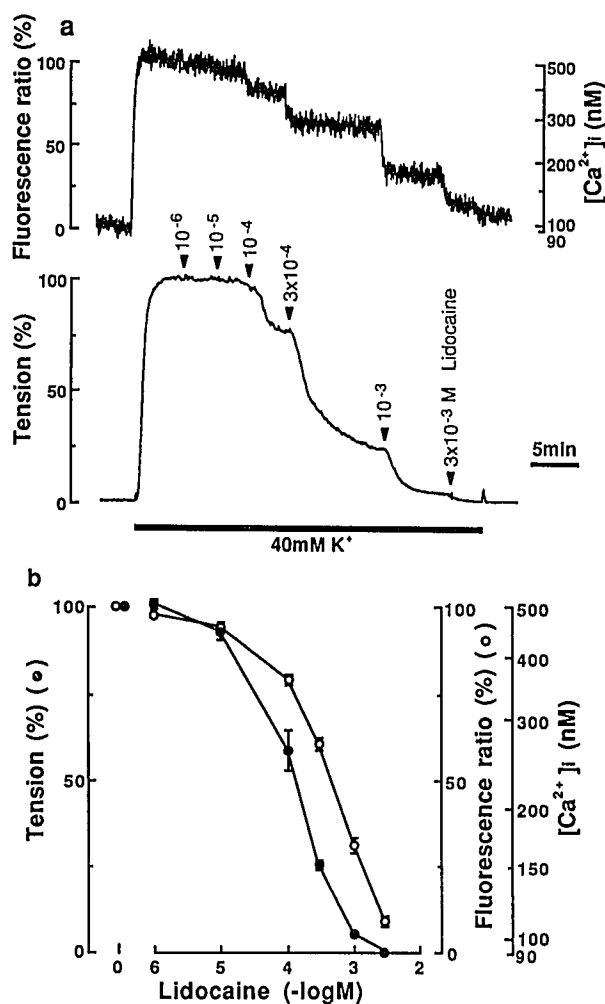
Normal PSS was of the following composition (in mM): NaCl 123, KCl 4.7,  $NaHCO_3$  15.5,  $KH_2PO_4$  1.2,  $MgCl_2$  1.2,  $CaCl_2$  1.25, and D-glucose 11.5. High  $K^+$  PSS was identical to normal PSS, except for an equimolar substitution of KCl for NaCl. The  $Ca^{2+}$ -free version of PSS was 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( $\beta$ -aminoethyl ether) $N,N,N',N'$ -tetra-

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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  $\pm$  SE ( $n$  = number of observations). For each observation, a



**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 ( $10^{-6}$ – $3 \times 10^{-3}$  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.

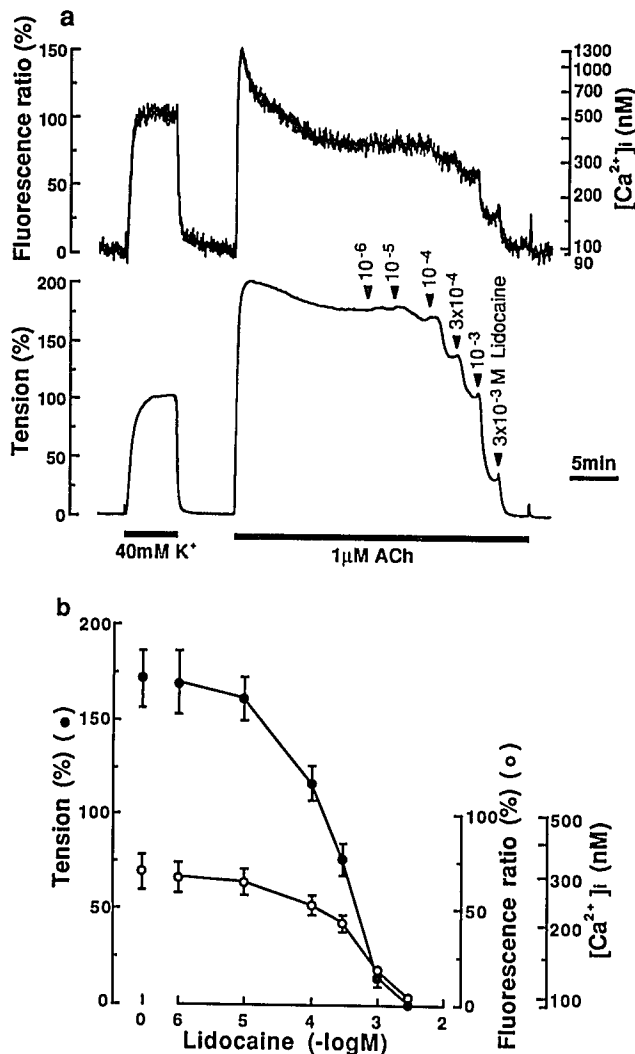
strip from a different animal was used. One-way analysis of variance for repeated measurements was used to determine the concentration-dependent effects. Two-way analysis of variance for repeated measurements was used to determine the statistical significance of lidocaine's effect on the contractions 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.*<sup>18</sup>

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

When lidocaine was cumulatively applied ( $10^{-6}$ – $3 \times 10^{-3}$  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  $3 \times 10^{-3}$  M lidocaine reduced  $[Ca^{2+}]_i$  and tension to  $9.2 \pm 1.7\%$  (110 nM) and  $0.4 \pm 0.2\%$ , respectively ( $n = 5$ ). The  $IC_{50}$  values (concentrations of lidocaine which induced 50% of the changes obtained with  $3 \times 10^{-3}$  M lidocaine) for  $[Ca^{2+}]_i$  and tension were approximately  $3.4 \times 10^{-4}$  M and  $1.2 \times 10^{-4}$  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 ( $10^{-6}$ – $3 \times 10^{-3}$  M) on elevated  $[Ca^{2+}]_i$  and tension induced by 1  $\mu$ M 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  $1 \mu M$  ACh. (A) Representative time course of the effect of cumulatively applied lidocaine ( $10^{-6}$ – $3 \times 10^{-3}$  M) on elevated  $[Ca^{2+}]_i$  and tension induced by  $1 \mu M$  ACh. (B) Concentration-dependent effect of lidocaine on elevated  $[Ca^{2+}]_i$  (○) and tension (●) induced by  $1 \mu M$  ACh. Lidocaine was cumulatively applied to the steady state of  $1 \mu M$  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  $3 \times 10^{-3}$  M lidocaine reduced  $[Ca^{2+}]_i$  and tension to  $4.0 \pm 1.0\%$  (99 nM) and  $0.6 \pm 0.4\%$ , respectively ( $n = 5$ ). The  $IC_{50}$  values (concentrations of lidocaine which induced 50% of the changes obtained with  $3 \times 10^{-3}$  M lidocaine) for  $[Ca^{2+}]_i$

and tension were approximately  $2.8 \times 10^{-4}$  M and  $1.5 \times 10^{-4}$  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  $-26.1 \pm 2.0\%$  (43 nM) at  $0$  mM extracellular  $Ca^{2+}$  to  $108.9 \pm 6.1\%$  (579 nM) at  $2.5$  mM extracellular  $Ca^{2+}$ , and tension increased from  $0 \pm 0\%$  to  $83.1 \pm 11.2\%$  ( $n = 10$ ). 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  $-15.8 \pm 1.4\%$  (60 nM) at  $0$  mM extracellular  $Ca^{2+}$  to  $73.4 \pm 6.3\%$  (328 nM) at  $5$  mM extracellular  $Ca^{2+}$ , and tension increased from  $0 \pm 0\%$  to  $33.2 \pm 13.2\%$  ( $n = 5$ ).

#### *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  $1 \mu M$  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  $-21.0 \pm 3.4\%$  (51 nM) at  $0$  mM extracellular  $Ca^{2+}$  to  $111.8 \pm 9.5\%$  (608 nM) at  $2.5$  mM extracellular  $Ca^{2+}$ , and tension increased from  $1.6 \pm 0.9\%$  to  $185.0 \pm 13.4\%$  ( $n = 5$ ). 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

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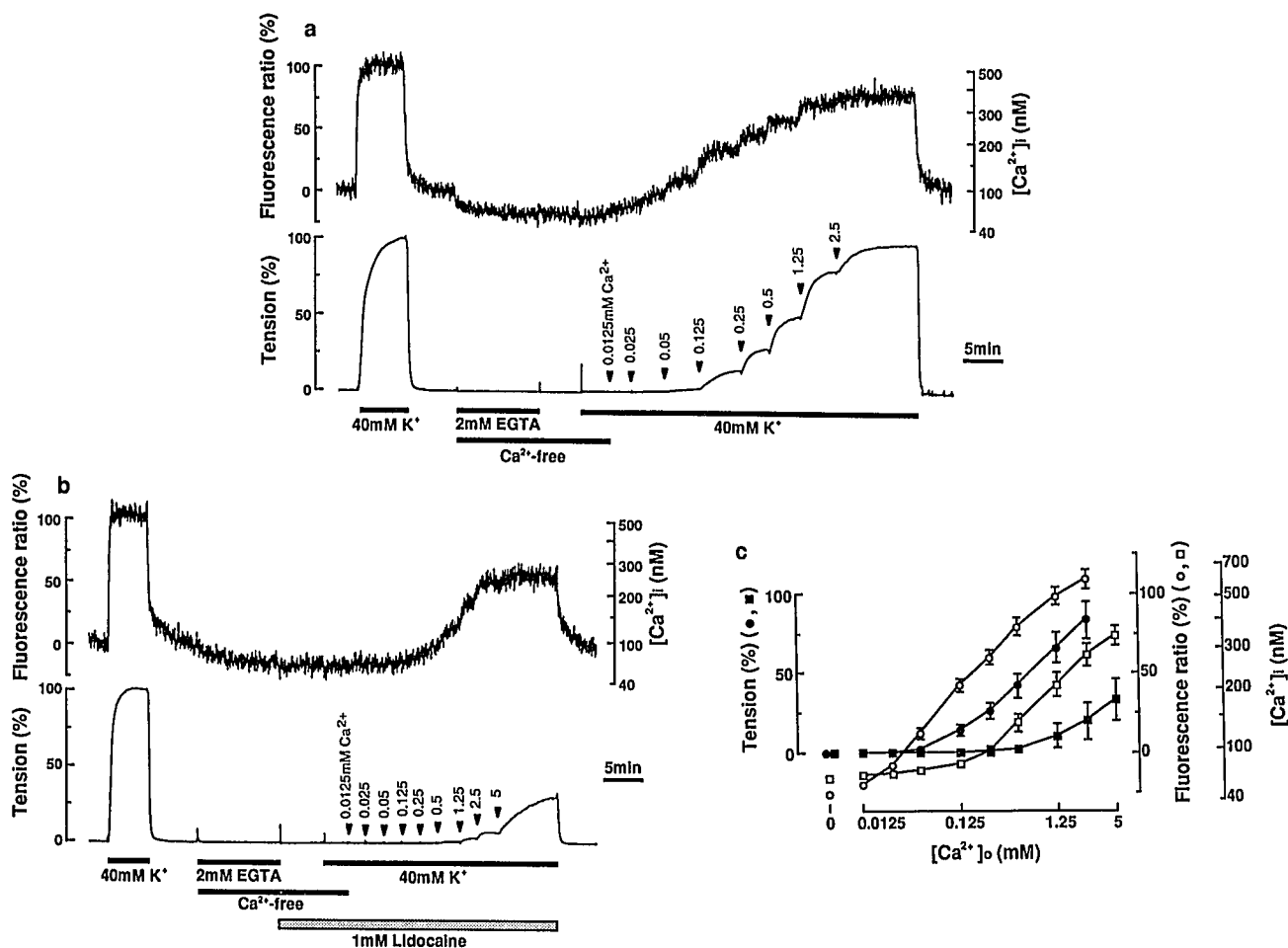


Fig. 4. Effect of 1 mM lidocaine on  $[Ca^{2+}]_i$  and tension development induced by cumulative application of external  $Ca^{2+}$  (0.0125–2.5 or 5 mM) during 40 mM  $K^+$  depolarization. (A) Representative time course of changes in  $[Ca^{2+}]_i$  and tension induced by the cumulative application 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  $[Ca^{2+}]_i$  ( $\square$ ) and tension development ( $\blacksquare$ ) induced by changes in extracellular  $Ca^{2+}$  during 40 mM  $K^+$  depolarization. Controls:  $[Ca^{2+}]_i$  ( $\circ$ ) and tension ( $\bullet$ ) 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  $-19.4 \pm 4.4\%$  (54 nM) at 0 mM extracellular  $Ca^{2+}$  to  $92.6 \pm 6.7\%$  (443 nM) at 5 mM extracellular  $Ca^{2+}$ , and tension increased from  $0.8 \pm 0.2\%$  to  $123.2 \pm 11.3\%$  ( $n = 5$ ).

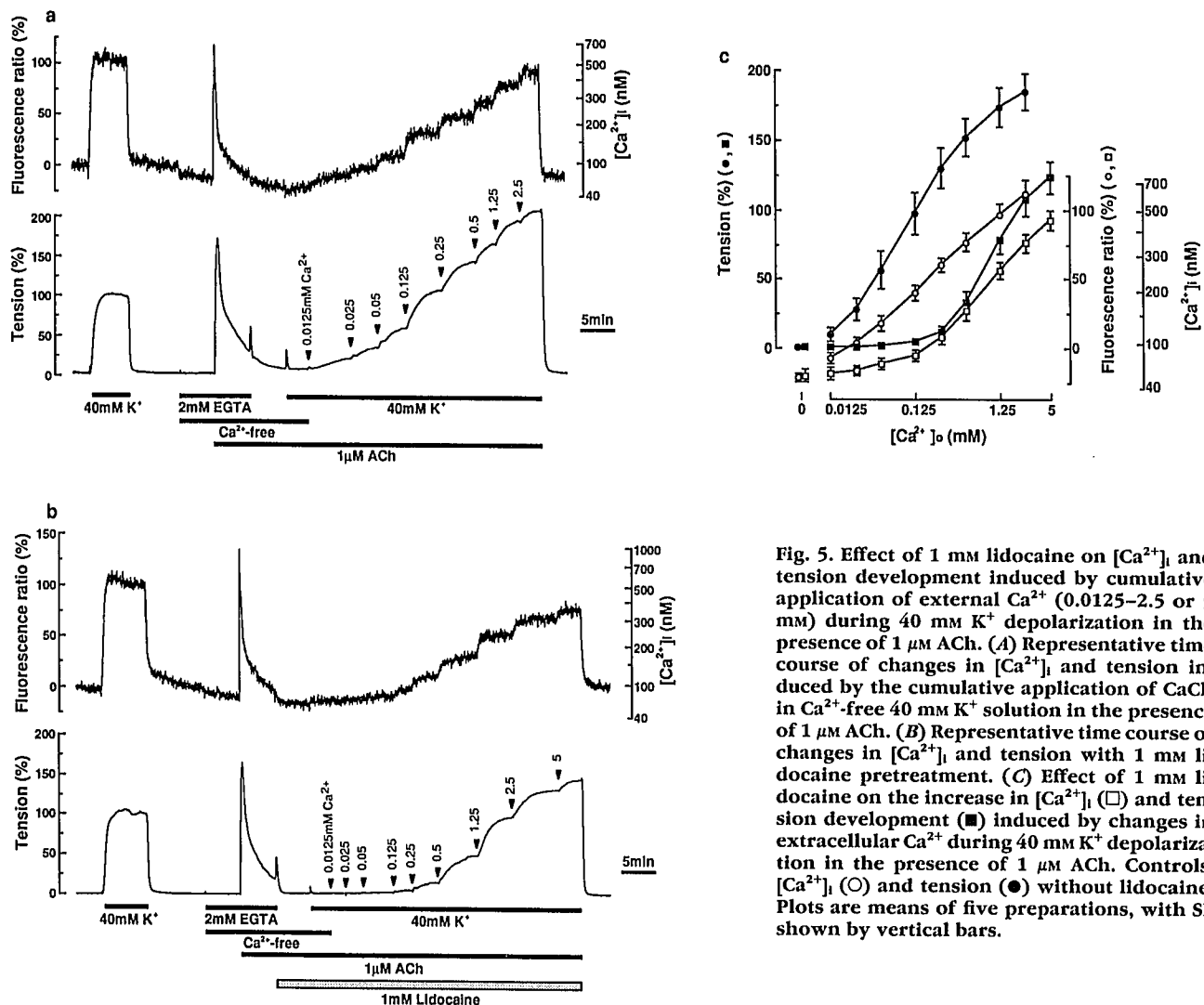
#### Effect of Lidocaine on $[Ca^{2+}]_i$ -Tension Relationship

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  $\mu$ M 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  $\mu$ M 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  $\mu$ M 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 appli-

cations of 1  $\mu$ M ACh in  $Ca^{2+}$ -free solution containing 2 mM EGTA. The application of 1  $\mu$ M 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

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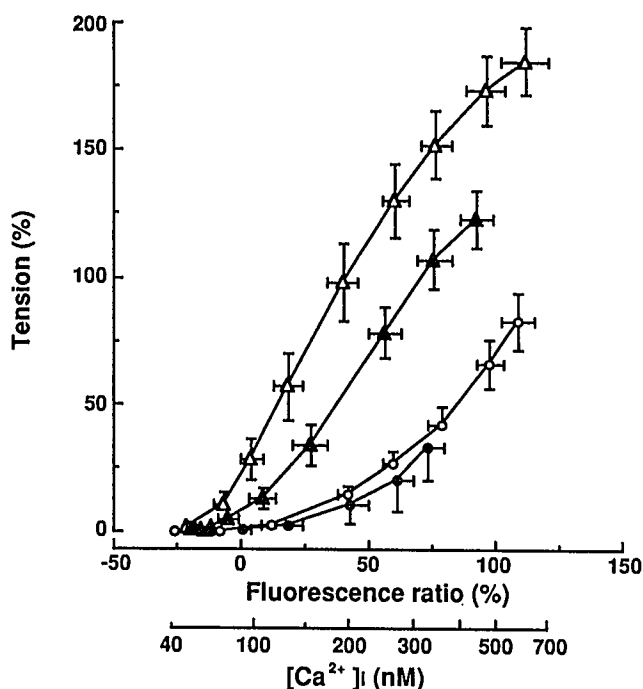


Fig. 6. Effect of lidocaine on the  $[Ca^{2+}]_i$ -tension relationship. The  $[Ca^{2+}]_i$ -tension relationship was obtained from data in figures 4 and 5.  $\circ$  and  $\bullet$  indicate values obtained from contractions induced by changes in extracellular  $Ca^{2+}$  during 40 mM  $K^+$  depolarization, with ( $\bullet$ ) or without ( $\circ$ ) treatment with 1 mM lidocaine.  $\Delta$  and  $\blacktriangle$  indicate values obtained from contractions induced by changes in extracellular  $Ca^{2+}$  during 40 mM  $K^+$  depolarization in the presence of 1  $\mu$ M ACh, with ( $\blacktriangle$ ) or without ( $\Delta$ ) 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).<sup>19</sup> 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, inosi-

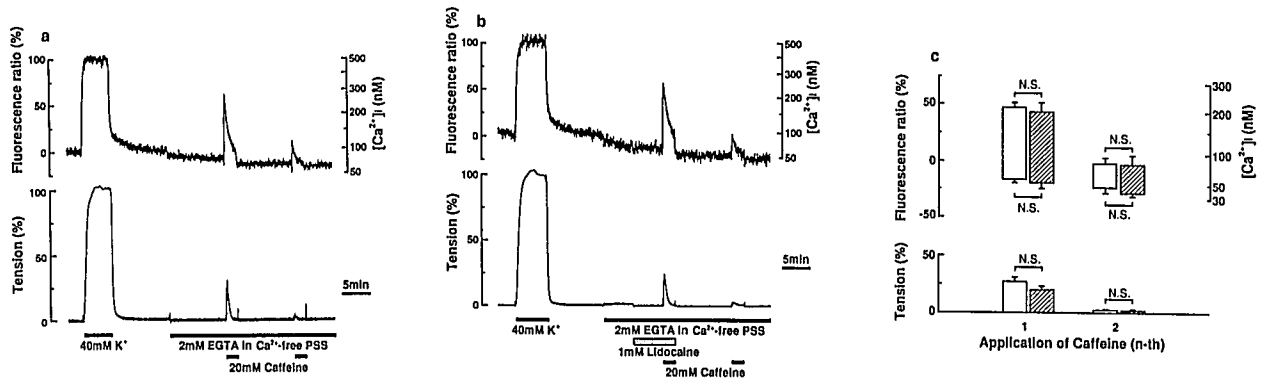
tol 1,4,5-trisphosphate ( $IP_3$ ) and 1,2-diacylglycerol (DAG).  $IP_3$  releases  $Ca^{2+}$  from sarcoplasmic reticulum. Through these two pathways, the increase in  $[Ca^{2+}]_i$  occurs.  $Ca^{2+}$  binds to calmodulin to activate myosin light chain kinase that catalyzes phosphorylation of the myosin light chain, activation of actomyosin ATPase, and contraction.<sup>20</sup> 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.<sup>21</sup> 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 ( $10^{-6} \sim 3 \times 10^{-3}$  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 ( $10^{-6} \sim 3 \times 10^{-3}$  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.*<sup>5</sup> reported that pretreatment with 7  $\mu$ M 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<sup>22</sup> 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<sup>23</sup> 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-





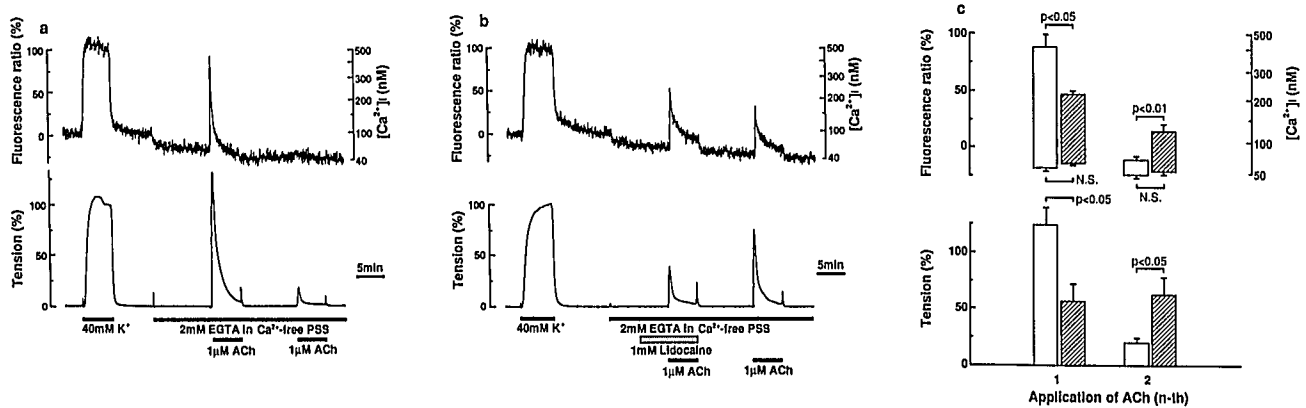
**Fig. 7.** Effect of 1 mM lidocaine on  $[Ca^{2+}]_i$  and tension development induced by 20 mM caffeine in the absence of extracellular  $Ca^{2+}$ . (A) Representative time course of  $[Ca^{2+}]_i$  and tension development induced by repeated applications of 20 mM caffeine in  $Ca^{2+}$ -free solution 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<sup>24</sup> reported that lidocaine decreased the Ca current in embryonic chick ventricular myocytes, in a concentration-dependent manner (10–1,000  $\mu M$ ). Also, Elliott<sup>25</sup> 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  $\mu M$ . 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  $\mu M$  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  $\mu M$  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  $\mu M$  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 ( $n = 4$ ).

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induced  $\text{Ca}^{2+}$  release mechanism, while ACh causes  $\text{Ca}^{2+}$  release by pharmacomechanical coupling mediated by  $\text{IP}_3$ .<sup>26</sup>

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

#### *Effects of Lidocaine on Myofilament $\text{Ca}^{2+}$*

##### *Sensitivity*

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

#### *Possible Site of Action of Lidocaine*

As already discussed, in pharmacomechanical coupling, DAG is produced together with  $\text{IP}_3$  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  $\text{Ca}^{2+}$  sensitivity of the contractile apparatus.<sup>32</sup> Lidocaine may inhibit both ACh-induced  $\text{IP}_3$  production and PKC activation, because, in the present study, lidocaine inhibited both the ACh-induced  $\text{Ca}^{2+}$  release and the ACh-induced increase in  $\text{Ca}^{2+}$  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.<sup>5</sup> 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<sup>33</sup> 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<sup>23</sup> that sodium salicylate,

which increases the negative surface charge of cell membranes, relaxed  $\text{Ca}^{2+}$ -induced contractions in  $\text{K}^+$ -depolarized guinea pig taenia pretreated with the cationic local anesthetics, including lidocaine, whereas salicylate did not affect the  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$ -induced contraction during  $\text{K}^+$  depolarization indicates that lidocaine may exert inhibitory effect on  $\text{Ca}^{2+}$  influx by its accumulation in cell membrane. Such an intramembranous location would be appropriate if lidocaine was to mediate effects on ACh-induced  $\text{Ca}^{2+}$  release and on ACh-induced increase of  $\text{Ca}^{2+}$  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  $\mu\text{g}/\text{ml}$ ,<sup>34</sup> that is, around 10–20  $\mu\text{M}$ . 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  $[\text{Ca}^{2+}]_i$  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  $\text{Ca}^{2+}$  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.<sup>2,3,35</sup> In addition to this central effect, the current study indicates that the inhibitory effect of lidocaine on myofilament  $\text{Ca}^{2+}$  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  $[\text{Ca}^{2+}]_i$ . In addition, lidocaine inhibits the ACh-induced increase of  $\text{Ca}^{2+}$  sensitivity of the contractile apparatus, although it has little effect on  $\text{Ca}^{2+}$  sensitivity during high  $\text{K}^+$  depolarization. The decrease in  $[\text{Ca}^{2+}]_i$  was attributed to inhibition of the influx of extracellular  $\text{Ca}^{2+}$ , as induced

by high  $\text{K}^+$  depolarization and by ACh, and to inhibition of the ACh-induced release of stored  $\text{Ca}^{2+}$ .

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#### References

1. Brandus V, Joffe S, Benoit CV, Wolff WI: Bronchial spasm during general anaesthesia. *Can Anaesth Soc J* 17:269–274, 1970
2. Downes H, Gerber N, Hirshman CA: I.V. lignocaine in reflex and allergic bronchoconstriction. *Br J Anaesth* 52:873–878, 1980
3. Downes H, Hirshman CA: Lidocaine aerosols do not prevent allergic bronchoconstriction. *Anesth Analg* 60:28–32, 1981
4. Weiss EB, Hargraves WA, Viswanath SG: The inhibitory action of lidocaine in anaphylaxis. *Am Rev Respir Dis* 117:859–869, 1978
5. Weiss EB, Anderson WH, O'Brien KP: The effect of local anesthetic, lidocaine, on guinea pig trachealis muscle *in vitro*. *Am Rev Respir Dis* 112:393–400, 1975
6. Downes H, Loehning RW: Local anesthetic contracture and relaxation of airway smooth muscle. *ANESTHESIOLOGY* 47:430–436, 1977
7. Okumura F, Denborough MA: Effects of anaesthetics on guinea pig tracheal smooth muscle. *Br J Anaesth* 52:199–204, 1980
8. Altura BM, Altura BT: Effects of local anesthetics, antihistamines, and glucocorticoids on peripheral blood flow and vascular smooth muscle. *ANESTHESIOLOGY* 41:197–214, 1974
9. Feinstein MB, Paimre M: Pharmacological action of local anesthetics on excitation-contraction coupling in striated and smooth muscle. *Fed Proc* 28:1643–1648, 1969
10. Fleisch JH, Titus E: Effect of local anesthetics on pharmacologic receptor systems of smooth muscle. *J Pharmacol Exp Ther* 186:44–51, 1973
11. Morgan JP, Morgan KG: Stimulus-specific patterns of intracellular calcium levels in smooth muscle of ferret portal vein. *J Physiol (Lond)* 351:155–167, 1984
12. Nishimura J, Kolber M, van Breemen C: Norepinephrine and GTP- $\gamma$ -S increase myofilament  $\text{Ca}^{2+}$  sensitivity in  $\alpha$ -toxin permeabilized arterial smooth muscle. *Biochem Biophys Res Commun* 157:677–683, 1988
13. Kobayashi S, Kitazawa T, Somlyo AV, Somlyo AP: Cytosolic heparin inhibits muscarinic and  $\alpha$ -adrenergic  $\text{Ca}^{2+}$  release in smooth muscle. *J Biol Chem* 264:17997–18004, 1989
14. Kodama M, Kanaide H, Abe S, Hirano K, Kai H, Nakamura M: Endothelin-induced Ca-independent contraction of the porcine coronary artery. *Biochem Biophys Res Commun* 160:1302–1308, 1989
15. Abe S, Kanaide H, Nakamura M: Front-surface fluorometry with fura-2 and effects of nitroglycerin on cytosolic calcium concentrations and tension in the coronary artery of the pig. *Br J Pharmacol* 101:545–552, 1990
16. Hirano K, Kanaide H, Abe S, Nakamura M: Effects of diltiazem on calcium concentrations in the cytosol and on force of contractions in porcine coronary arterial strips. *Br J Pharmacol* 101:273–280, 1990
17. Grynkiewicz G, Poenie M, Tsien RY: A new generation of  $\text{Ca}^{2+}$  indicators with greatly improved fluorescence properties. *J Biol Chem* 260:3440–3450, 1985
18. De Lean A, Munson PJ, Rodbard D: Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand

## MECHANISMS OF LIDOCAINE-INDUCED AIRWAY RELAXATION

assay, and physiological dose-response curves. *Am J Physiol* 235: E97-E102, 1978

19. Coburn RF, Baron CB: Coupling mechanisms in airway smooth muscle. *Am J Physiol* 258:L119-L133, 1990

20. Kamm KE, Stull JT: The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. *Annu Rev Pharmacol Toxicol* 25:593-620, 1985

21. Somlyo AP, Kitazawa T, Kobayashi S, Gong MC: Pharmacomechanical coupling: The membranes talk to the crossbridges, Regulation of Smooth Muscle Contraction. Edited by Moreland RS. New York, Plenum Press, 1991, pp 185-208

22. Hay DWP, Wadsworth RM: Local anaesthetic activity of organic calcium antagonists: Relevance to their actions on smooth muscle. *Eur J Pharmacol* 77:221-228, 1982

23. Spedding M, Berg C: Antagonism of  $Ca^{2+}$ -induced contractions of  $K^{+}$ -depolarized smooth muscle by local anaesthetics. *Eur J Pharmacol* 108:143-150, 1985

24. Josephson IR: Lidocaine blocks Na, Ca and K currents of chick ventricular myocytes. *J Mol Cell Cardiol* 20:593-604, 1988

25. Elliott P: Action of antiepileptic and anaesthetic drugs on Na and Ca-spikes in mammalian non-myelinated axons. *Eur J Pharmacol* 175:155-163, 1990

26. van Breemen C, Saida K: Cellular mechanisms regulating  $[Ca^{2+}]_i$  smooth muscle. *Annu Rev Physiol* 51:315-329, 1989

27. Gerthoffer WT, Murphey KA, Gunst SJ: Aequorin luminescence,

myosin phosphorylation, and active stress in tracheal smooth muscle. *Am J Physiol* 257:C1062-C1068, 1989

28. Ozaki H, Kwon SC, Tajimi M, Karaki H: Changes in cytosolic  $Ca^{2+}$  and contraction induced by various stimulants and relaxants in canine tracheal smooth muscle. *Pflügers Arch* 416:351-359, 1990

29. Yamakage M: Direct inhibitory mechanisms of halothane on canine tracheal smooth muscle contraction. *ANESTHESIOLOGY* 77:546-553, 1992

30. Tanaka T, Hidaka H: Interaction of local anesthetics with calmodulin. *Biochem Biophys Res Commun* 101:447-453, 1981

31. Nosaka S, Kamaya H, Ueda I, Wong KC: Smooth muscle contraction and local anesthetics: Calmodulin-dependent myosin light-chain kinase. *Anesth Analg* 69:504-510, 1989

32. Rasmussen H, Takawa Y, Park S: Protein kinase C in the regulation of smooth muscle contraction. *FASEB J* 1:177-185, 1987

33. Burdyga ThV, Magura IS: The effects of local anaesthetics on the electrical and mechanical activity of the guinea-pig ureter. *Br J Pharmacol* 88:523-530, 1986

34. Gianelly R, von der Groeben JO, Spivack AP, Harrison DC: Effect of lidocaine on ventricular arrhythmias in patients with coronary heart disease. *N Engl J Med* 277:1215-1219, 1967

35. Downes H, Hirshman CA, Leon DA: Comparison of local anesthetics as bronchodilator aerosols. *ANESTHESIOLOGY* 58:216-220, 1983