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The mechanism for the contraction induced by leukotriene C4 in guinea-pig taenia coli

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1 The mechanism underlying the LTC_4-induced contraction of guinea-pig taenia coli was determined using the simultaneous measurements of $[Ca^{2+}]_i$ and force in whole muscle preparations. Additional experiments were performed in receptor coupled permeabilized preparation. For comparison purposes, the contraction which was induced by a typical G-protein mediated agonist, carbachol was also characterized.

2 LTC_4 induced a contraction in the guinea-pig taenia coli in a concentration-dependent manner. The maximal response was obtained at 100 nM and the EC_{50} value was 5.4 ± 1.9 nM.

3 Both LTC_4 and carbachol induced increases in $[Ca^{2+}]_i$ and force. The maximum force induced by 100 nM LTC_4 was significantly smaller than that induced by 10 μM carbachol, although an increase in $[Ca^{2+}]_i$ produced by both agonists was similar. In the permeabilized preparations, carbachol, but not LTC_4, induced an additional force development at a fixed Ca^{2+} concentration.

4 LTC_4 induced no increase in $[Ca^{2+}]_i$ and force in the Ca^{2+}-free solution, while carbachol induced transient increases in both $[Ca^{2+}]_i$ and force in a Ca^{2+}-free solution.

5 Both diltiazem and SK&F 96365 significantly inhibited the LTC_4- and carbachol-induced increases in $[Ca^{2+}]_i$ and force in normal PSS. The inhibitory pattern of $[Ca^{2+}]_i$ by these drugs was also similar.

6 We thus conclude that LTC_4 induces the contraction of the guinea-pig taenia coli mainly through Ca^{2+} influx *via* both the diltiazem-sensitive and SK&F 96365-sensitive Ca^{2+} channels, without affecting either the Ca^{2+}-sensitivity or the intracellular Ca^{2+} release. These results indicated that the mechanism underlying the LTC_4-induced contraction differs greatly from that for conventional G-protein mediated agonists, such as carbachol.

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Keywords: Leukotriene C_4; carbachol; Ca^{2+} channel; Ca^{2+} sensitivity

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium; CSS, cytoplasmic substitution solution; EGTA, ethyleneglycol-bis (β-aminoethyl-ether)-N,N,N′,N′,-tetraacetic acid; fura-PE3/AM, fura-PE3 acetoxymethyl ester; GTP, guanosine 5′-triphosphate; LTC_4, leukotriene C_4; PSS, physiological salt solution; TTX, tetrodotoxin; VOC, voltage-operated Ca^{2+} channel; 0.3 G, 0.3 mM ethyleneglycol-bis (β-aminoethylether)-N, N, N′, N′,-tetraacetic acid

Introduction

Leukotriene C_4 (LTC_4) is one of the sulphidopeptide leukotrienes and belongs to the family of cysteinyl leukotrienes, which are the major metabolites of arachidonic acid (Jonsson, 1998). LTC_4 is known as a slow-reacting substance of anaphylaxis (SRSA) and was first identified in mouse mastcytoma cells (Murphy *et al*., 1979). LTC_4 is released from inflammatory cells, such as mast cells (Barrett *et al*., 1986; Heavey *et al*., 1988) and plays an important role as a potent mediator of allergic reaction. This compound was demonstrated to be released after the stimulation by the antigen in various human and animal tissues and its synthesis has also been shown to be associated with such inflammatory diseases as bronchial asthma, psoriasis, rheumatoid arthritis, and inflammatory bowel diseases (Henderson, 1994). Elevated LTC_4 induces a contraction of the smooth muscle, such as the airway, gastrointestinal and vascular smooth muscle (Allen *et al*., 1998; Dahlen *et al*., 1986; Freedman *et al*., 1993).

In gastrointestinal smooth muscle, LTC_4 plays an important role in various pathological states, such as intestinal anaphylactic reaction (persistent diarrhoea, vomiting, and dehydration), necrotizing enterocolitis, ulcerative colitis, ischaemia-reperfusion injury (Anadol *et al*., 1998; Dollberg *et al*., 1992; Hsueh *et al*., 1986; Ohtsuka *et al*., 1998). An increased contractility of the visceral smooth muscle might be involved in the pathogenesis of these gastrointestinal diseases. However, the mechanism for the LTC_4-induced contraction of the visceral smooth muscle has not yet been fully elucidated. A previous study indicated that LTC_4-induced contraction of guinea-pig taenia coli was mainly dependent on the Ca^{2+} influx through the voltage-operated Ca^{2+} channel (VOC) (Zschauer *et al*., 1988).

On the other hand, the mechanism for the contraction of the visceral smooth muscle induced by such muscarinic

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agonists, as acetylcholine and carbamylcholinechloride (carbachol), has been extensively investigated (Bolton & Zholos, 1997). For example, carbachol has been shown to induce phasic contraction in guinea-pig taenia coli by increasing $[Ca^{2+}]_i$ as well as by increasing Ca^{2+} sensitivity (Otto *et al*., 1996). Increases in $[Ca^{2+}]_i$ induced by carbachol are caused by both intracellular Ca^{2+} release and Ca^{2+} influx from the extracellular space (Otto *et al*., 1996; Wang *et al*., 1997).

In the present study, we determined the mechanism for the LTC_4-induced contraction of guinea-pig taenia coli and compared it with that for carbachol-induced contraction. For this purpose, we carried out simultaneous measurements of $[Ca^{2+}]_i$ and the force induced by either LTC_4 or carbachol. We also investigated the effect of LTC_4 or carbachol on the Ca^{2+} sensitivity using a receptor-coupled permeabilized preparation. The results obtained indicate that the mechanism for LTC_4-induced contraction was markedly different from that for carbachol-induced contraction.

Methods

Tissue preparation

These protocols were approved by the Animal Care and the Committee of the Research Institute of Angiocardiology, Graduate School of Medical Sciences, Kyushu University. Male Hartley guinea-pigs weighing 350–400 g were used throughout. The guinea-pigs were sacrificed by bleeding under deep anaesthesia with ether. After the taenia coli was isolated, the mucosa and the circumference muscle layer were removed with the aid of a binocular microscope. Next, the longitudinal muscle tissue preparation was cut into strips ($1 \times 4 \times 0.5$ mm).

Measurement of force development

Strips of taenia coli were mounted vertically in a quartz organ bath. One end of the strips was connected to a fixed hook, while the other end was connected to a strain gauge (TB-612-T, Nihon Koden, Japan). The strip was stimulated with 60 mM K^+ depolarization every 15 min with a stepwise increase in the resting load until the maximal response was obtained. The length at the optimum resting load so obtained was about 1.5 times longer than the initial length. All experimental procedure were performed in the presence of 1 μM tetrodotoxin (TTX). The presence of 1 μM TTX did not affect the force development to 60 mM K^+. The developed force in response to agonists was expressed as a percentage, assigning the values obtained at rest in normal PSS (5.9 mM K^+) and at the steady state of contraction induced by 60 mM K^+ PSS to be 0 and 100%, respectively.

Fura-PE3 loading

The guinea-pig taenia coli strips were loaded with the Ca^{2+} indicator dye, fura-PE3 in the form of acetoxymethyl ester (fura-PE3/AM), and then were incubated in oxygenated (a mixture of 95% O_2 and 5% CO_2) Dulbecco's modified Eagle medium (DMEM) containing 50 μM fura-PE3/AM dissolved in dimethylsulphoxide and 5% foetal bovine serum for 8 h at 37°C (containing 0.02% Pluronic F-127). After loading with fura-PE3/AM, the strips were rinsed in normal PSS to remove the dye remaining in the extracellular space and then were equilibrated in normal PSS for about 1 h before starting the experimental protocols.

Front-surface flouorometry

The changes in the fluorescence intensity of Ca^{2+}-fura-PE3 complex of the strips of taenia coli were monitored with a front-surface fluorometer as previously reported (Hirano *et al*., 1990). The fluorescence intensities at 340 and 380 nm excitation were monitored and their ratio (F340/F380) were recorded as an indicator of $[Ca^{2+}]_i$ at 37°C. Before starting each experimental protocol, the response to 60 mM K^+ depolarization was recorded as a reference response. Any changes in the fluorescence ratio were expressed as a percentage, assigning values in normal PSS (5.9 mM K^+) and at the steady state contraction induced by 60 mM K^+ depolarization to be 0 and 100%, respectively.

Permeabilization of taenia coli strips with α-toxin

Permeabilization of guinea-pig taenia coli was performed according to the methods described previously with minor modifications (Niiro *et al*., 1998; Nishimura *et al*., 1988). Guinea-pig taenia coli were cut into small strips (about 0.5 mm in width and 1 mm in length). Permeabilization was accomplished by incubating the small strips with staphylococcal α-toxin (5000 units ml^{-1}) for 60 min in a Ca^{2+}-free cytoplasmic substitution solution (CSS) containing 10 mM EGTA at room temperature. After permeabilization, the strips were mounted between two tungsten wires, one of which was fixed to the desk and the other was attached to a force transducer (UL2; Minebea Co., Japan). The force development in the α-toxin permeabilized tissue was determined at room temperature. Force in Ca^{2+}-free CSS containing 10 mM EGTA and those induced by 10 μM Ca^{2+} were designated to be 0 and 100%, respectively. The CSS containing indicated concentration of free Ca^{2+} was prepared as previously described (Zhou *et al*., 1999), using the EGTA-Ca^{2+} binding constant of 10^6 M^{-1} (Saida & Nonomura, 1978).

Solutions and chemicals

The composition of the normal (5.9 mM K^+) PSS was as follows (in mM): NaCl 123, KCl 4.7, $NaHCO_3$ 15.5, $KHPO_4$ 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, and D-glucose 11.5. Hyperosmotic 60 mM K^+ solution was prepared by addition of KCl in the normal PSS, because almost all the maximum reproducible responses of force development to high K^+ were obtained at this concentration. A Ca^{2+}-free solution was made by omitting $CaCl_2$ from normal solution and adding EGTA (0.3 mM). PSS was gassed with a mixture of 5% CO_2 and 95% O_2, with the resulting pH being 7.4.

LTC_4 was purchased from Cayman Chemical (Ann Arber, MI, U.S.A.). Fura PE3/AM was purchased from Texas Fluorescence Laboratory (Austin, TX, U.S.A.). DMEM was purchased from Gibco (Grand Island, NY, U.S.A.). Carbamylcholinechloride (carbachol) and α-toxin were purchased from Sigma Chemical (St, Louis, MO, U.S.A.). Tetrodotoxin (TTX) was purchased from Wako (Osaka,

Japan), and EGTA was purchased from Dojindo (Kumamoto, Japan).

Data analysis

All data from the simultaneous measurements of $[Ca^{2+}]_i$ and force were collected using a computerized data acquisition system (MacLab; Analog Digital instruments, Castle Hill, Australia: Macintosh, Apple Computer, Cupertino, CA, U.S.A.). The representative traces shown were directly printed from the data obtained using this system. All data are the mean ± s.e.mean (n = number of the experiments). A strip obtained from one animal was used for each experiment, therefore the n value indicates the number of animals. A statistical analysis was performed using the unpaired Student's t-test, and P values of less than 0.05 were considered to be statistically significant.

Results

Effect of LTC_4 on the force development of guinea-pig taenia coli

As shown in Figure 1, LTC_4 at a concentration of 10 nM in the normal PSS induced a sustained contraction (Figure 1d), while lower concentrations of LTC_4 induced oscillatory contractions with or without an initial transient contraction (Figure 1b, c). We therefore evaluated the LTC_4-induced contraction by the area under the percentage force curve, for the construction of the concentration-response curve. The contractile responses for 15 min after the application of LTC_4 were quantified by digitizing force traces using the NIH image 1.61. The relative value of the force development was expressed as a percentage, assigning the values of the area under the curve for 15 min induced by 60 mM K^+ to be 100%. The concentration-response curve so obtained is shown in Figure 1e. LTC_4 (10 pM – 100 nM) caused a concentration-dependent contraction. A maximum response was obtained at 100 nM and the EC_{50} value was 5.4 ± 1.9 nM (n = 5).

Change in the $[Ca^{2+}]_i$ and force development induced by LTC_4 and carbachol

Figure 2a, d show representative recordings of the changes in $[Ca^{2+}]_i$ and force development induced by 100 nM LTC_4 and 10 μM carbachol in normal PSS, respectively. The relative value of fluorescence and force was expressed as a percentage, assigning the values in normal (5.9 mM K^+) and control 60 mM K^+ PSS to be 0 and 100%, respectively. When each strip was exposed to 100 nM LTC_4 or 10 μM carbachol, $[Ca^{2+}]_i$ rose rapidly and reached a peak in 1.7 min and 11 s, respectively, and then slowly declined until reaching a steady state level, with a similar time course. The peak levels of the $[Ca^{2+}]_i$ elevations induced by 100 nM LTC_4 and 10 μM carbachol were not significantly different (141.5 ± 15.0%; n = 5 and 141.4 ± 6.2%; n = 15, respectively). The developed force induced by 10 μM carbachol rose rapidly and reached a peak in 25 s, while that of LTC_4 rose more slowly than that of carbachol and reached a peak in 4.4 min. The maximum force induced by 100 nM LTC_4 (152.8 ± 15.8%; n = 5) was significantly smaller than that induced by 10 μM carbachol (221.3 ± 15.0%; n = 15), although the rise in $[Ca^{2+}]_i$ was similar for both. Figure 2b, c, e, f show the average time courses of the change in $[Ca^{2+}]_i$ and the force development during the contractions induced by 100 nM LTC_4 and 10 μM carbachol. It is clear that the force development induced by 100 nM LTC_4 was smaller than that induced by 10 μM carbachol at each time point, while the rise in $[Ca^{2+}]_i$ was similar to that of each other at each time point.

Effect of LTC_4 and carbachol on the $[Ca^{2+}]_i$-force relationships determined in the intact preparation

To determine the effect of LTC_4 and carbachol on the Ca^{2+} sensitivity of the contractile apparatus of the guinea-pig taenia coli, we first determined the basic $[Ca^{2+}]_i$-force relationship by monitoring the change in $[Ca^{2+}]_i$ and force induced by the cumulative applications of extracellular Ca^{2+} during the stimulations with 60 mM K^+ depolarization. As shown in Figure 3a, after recording the response of the control contraction, strips were first exposed to the Ca^{2+}-free PSS containing 0.3 mM EGTA for 10 min and then to the Ca^{2+}-free PSS without EGTA for 5 min before the stimulations with Ca^{2+}-free 60 mM K^+-depolarization. When extracellular Ca^{2+} was cumulatively applied from 0 to 2.5 mM in a stepwise manner, the graded elevations of $[Ca^{2+}]_i$ and force were observed. During 60 mM K^+ depolarization, $[Ca^{2+}]_i$ and force increased to 117.0 ± 7.0% and 92.5 ± 4.3% (n = 5), respectively, at the extracellular Ca^{2+} concentration of 2.5 mM. We used the data in Figure 3a to plot the basic $[Ca^{2+}]_i$-force relation curve of the Ca^{2+}-induced contraction.

Figure 3b shows the $[Ca^{2+}]_i$-force relation curve during contractions induced by 100 nM LTC_4 using the data points shown in Figure 2b, c. The basic $[Ca^{2+}]_i$-force relationship curve was also plotted for comparison purposes. The $[Ca^{2+}]_i$-force curve during activation by 100 nM LTC_4 was located at almost the same position as that of the basic $[Ca^{2+}]_i$-force curve. Similarly, the $[Ca^{2+}]_i$-force relation curve during the contractions induced by 10 μM carbachol was plotted (Figure 3c), using the data points shown in Figure 2e, f. In contrast to LTC_4, the $[Ca^{2+}]_i$-force curve during contraction by 10 μM carbachol was located higher and more to the left than the basic $[Ca^{2+}]_i$-force curve. These results indicated that carbachol, but not LTC_4, induced the increase in the Ca^{2+} sensitivity of the contractile apparatus.

Effects of LTC_4 and carbachol on the force development in α-toxin permeabilized preparations

To further confirm that carbachol, but not LTC_4, induces the increase in the Ca^{2+} sensitivity of the contractile apparatus, we carried out experiments using permeabilized preparation. As shown in Figure 4, the application of 100 nM LTC_4, after a steady state contraction had been induced by a mixture of 1 μM Ca^{2+} and 10 μM guanosine 5′-triphosphate (GTP), caused a small additional force development at a constant $[Ca^{2+}]_i$. In contrast, the application of 10 μM carbachol caused a large additional force development at a constant $[Ca^{2+}]_i$ level under the same conditions.

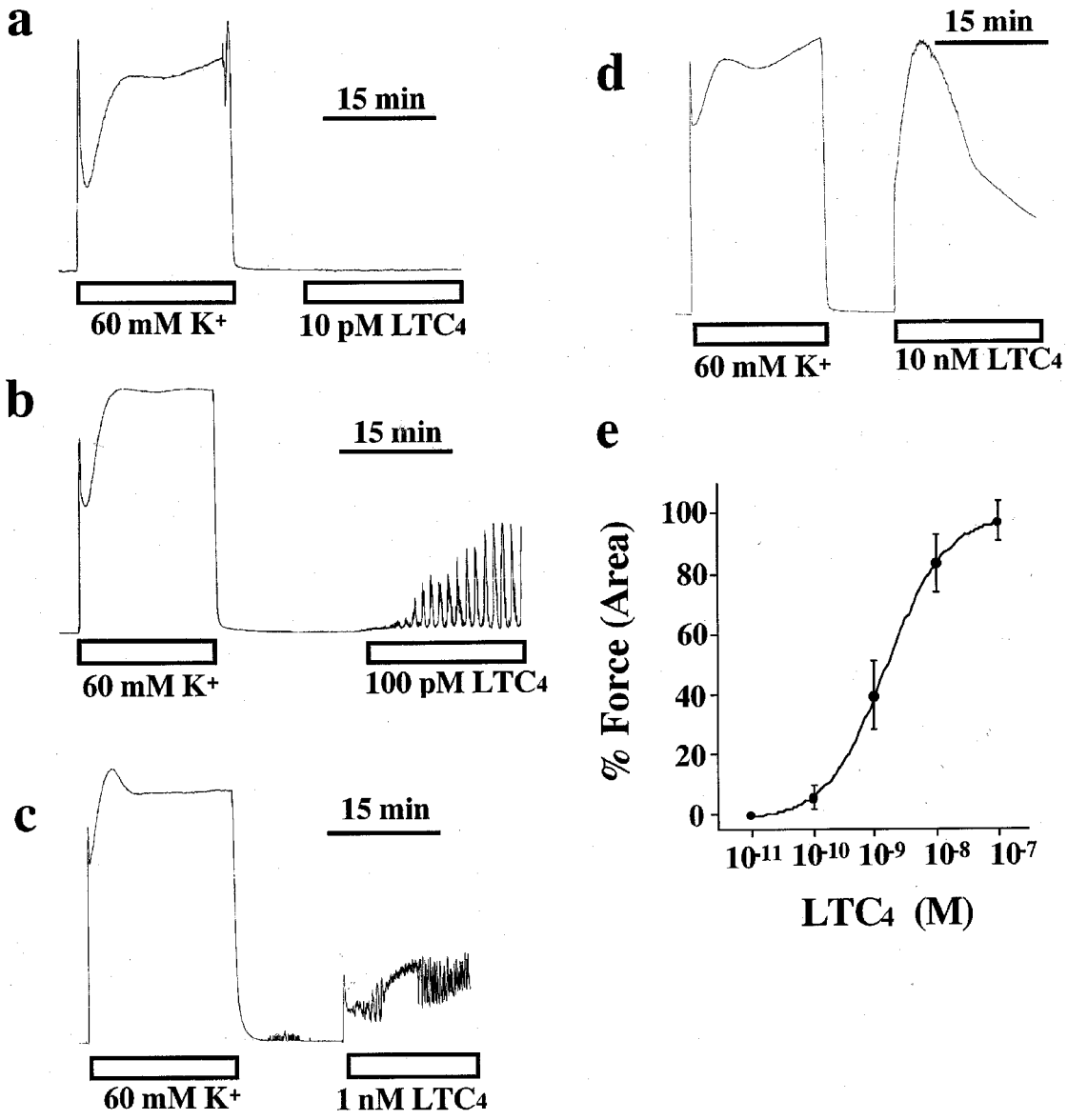

Figure 1 Representative recordings of contractions in guinea-pig taenia coli induced by various concentrations of LTC_4 (a: 10 pM, b: 100 pM, c: 1 nM, d: 10 nM) in normal PSS. (e) Concentration-response relationship of the LTC_4-induced contraction of guinea-pig taenia coli. The contractile responses were quantitated by digitizing force traces using the NIH image 1.61. The concentration-response curve for LTC_4 was constructed using the calculated area under the curve. The relative value of the force development was expressed as a percentage, assigning the values of the area under the curve for 15 min induced by 60 mM K^+ to be 100%. Data are expressed as the means $\pm$ s.e.mean ($n=5-8$).

Effects of removal of extracellular Ca^{2+} with EGTA on the LTC_4 and carbachol induced increases in $[Ca^{2+}]_i$ and force of guinea-pig taenia coli

To clarify the effects of LTC_4 and carbachol on the Ca^{2+} release from the intracellular store, we applied 100 nM LTC_4 or 10 μM carbachol in the Ca^{2+}-free PSS containing 0.3 mM EGTA. When the strip was exposed to Ca^{2+}-free PSS containing 0.3 mM EGTA, $[Ca^{2+}]_i$ decreased to $-38.9\pm2.7\%$ ($n=10$) in 3 min without affecting the resting tension. At this point, depolarization with 60 mM K^+ induced no increase in either the $[Ca^{2+}]_i$ or force, thus indicating that extracellular Ca^{2+} was completely chelated by EGTA while, in addition, no Ca^{2+}-influx was observed (data not shown) (Nasu & Zwai, 1997). In a Ca^{2+}-free PSS containing 0.3 mM EGTA, 100 nM LTC_4 did not induce any transient $[Ca^{2+}]_i$ (Figure 5a). On the other hand, as shown in Figure 5b, 10 μM carbachol induced a small amount of transient $[Ca^{2+}]_i$ ($-7.1\pm6.5\%$, $n=10$) accompanied by large transient contraction ($152.4\pm13.2\%$ $n=10$). Figure 5c shows the changes in $[Ca^{2+}]_i$ and tension induced by 10 μM carbachol in a Ca^{2+}-free PSS containing 100 nM LTC_4. The peak levels of $[Ca^{2+}]_i$ and contraction observed in this protocol were $-6.2\pm4.2\%$ ($n=7$) and $167.8\pm18.9\%$ ($n=7$), respectively. As shown in Figure 5d, there was no difference

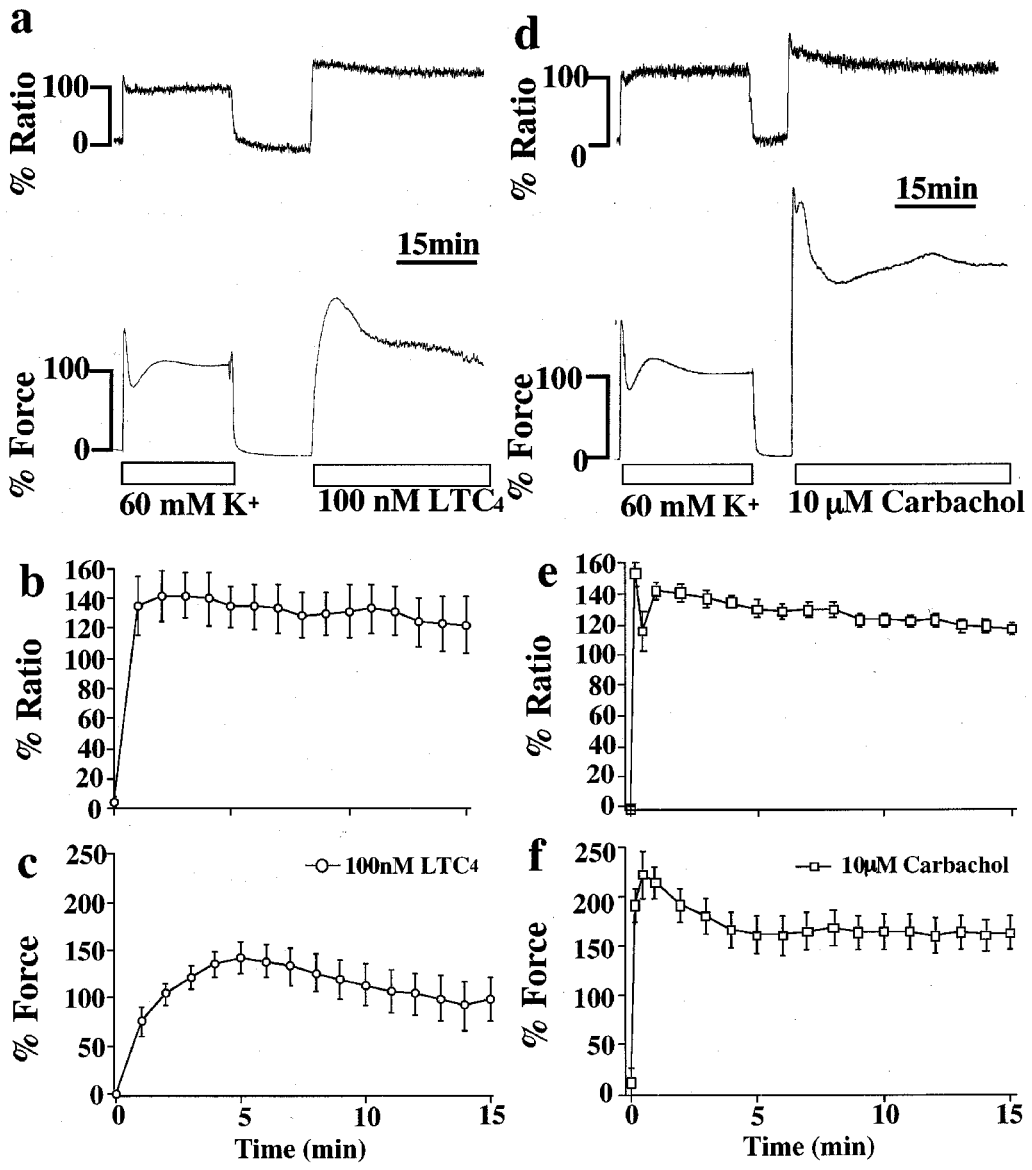

Figure 2 The time courses of the changes in the fluorescence ratio ($[Ca^{2+}]_i$) and the force development induced by LTC_4 and carbachol. (a) A representative recording showing the changes in $[Ca^{2+}]_i$ and force induced by 100 nM LTC_4 in normal PSS. The level of $[Ca^{2+}]_i$ and force at rest (5.9 mM K^+-PSS) and at the steady state of contraction induced by 60 mM K^+ depolarization were assigned to be 0 and 100%, respectively. (b and c) A summary of the time course of the changes in the $[Ca^{2+}]_i$ and force induced by 100 nM LTC_4 obtained from five similar independent experiments using five different guinea-pigs. (d) A representative recording showing the changes in $[Ca^{2+}]_i$ and force induced by 10 μM carbachol in normal PSS. (e and f) A summary of the time course of the changes in $[Ca^{2+}]_i$ and force induced by 10 μM carbachol ($n=5$). The vertical bars represent s.e.mean.

in the changes in $[Ca^{2+}]_i$ and force induced by carbachol in Ca^{2+}-free PSS between the presence and absence of LTC_4.

Effects of diltiazem and SK&F 96365 on the increases in $[Ca^{2+}]_i$ and force development induced by LTC_4 and carbachol in the presence of extracellular Ca^{2+}

In order to examine the Ca^{2+} influx pathways, two different types of Ca^{2+} channel antagonist, diltiazem (a L-type Ca^{2+} channel blocker) and SK&F 96365 were applied 10 min before and during activation by 100 nM LTC_4 and 10 μM carbachol. In order to evaluate the effect of these drugs on the Ca^{2+} influx pathways, comparisons were made during the sustained phase of contraction (15 min after the stimulation). As shown in Figure 6a, b, pretreatment with 10 μM diltiazem almost completely inhibited the maintained contraction in both LTC_4 and carbachol stimulation, while it did not inhibit the increase in $[Ca^{2+}]_i$ completely in either case. The $[Ca^{2+}]_i$-force relationship in both LTC_4 and carbachol stimulations of diltiazem pretreated preparations were similar to the basic $[Ca^{2+}]_i$-force relationships observed during 60 mM K^+-depolarization, as shown in Figure 3b or c. SK&F 96365 (10 μM) also inhibited the increases in $[Ca^{2+}]_i$ and force in a similar pattern to diltiazem, although the maintained force was more resistant to this drug than to diltiazem (traces not shown). When pretreatment was done by 10 μM diltiazem

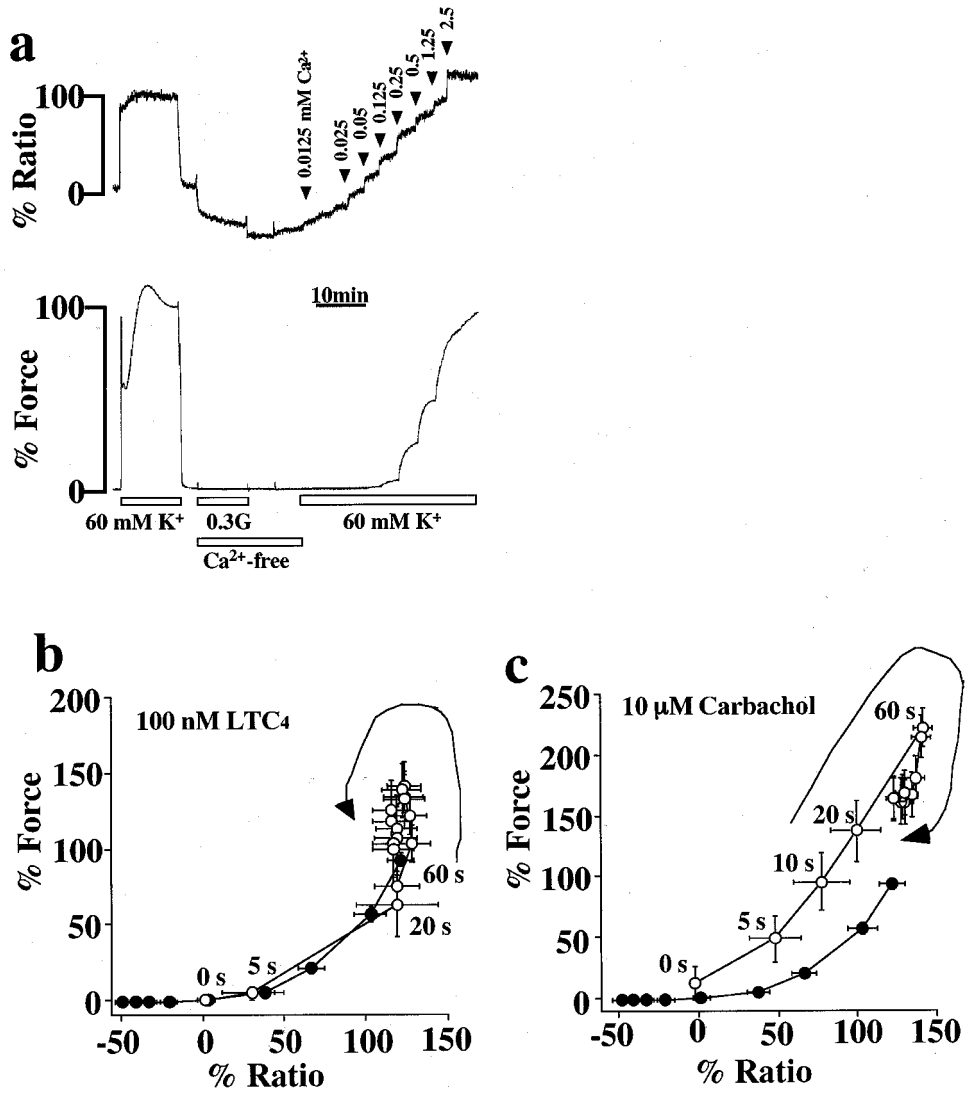

Figure 3 Changes in $[Ca^{2+}]_i$ and force development induced by the cumulative application of extracellular Ca^{2+} during 60 mM K^+-depolarization, and the temporal changes in the $[Ca^{2+}]_i$-force relationship during contraction induced by LTC_4 and carbachol. (a) A representative recording showing the changes in $[Ca^{2+}]_i$ and force induced by the cumulative applications of extracellular Ca^{2+} during the 60 mM K^+ depolarization. After exposure to 0.3 mM EGTA-containing the Ca^{2+}-free medium for 10 min and to the Ca^{2+}-free media without EGTA for 5 min, the strips were stimulated with 60 mM K^+-depolarization in the Ca^{2+}-free media, and then the extracellular Ca^{2+} was increased in a stepwise manner from 0–2.5 mM. The numbers noted by the arrow heads indicate the final concentration of extracellular Ca^{2+} at each step. The level of $[Ca^{2+}]_i$ and force at rest (5.9 mM K^+-PSS) and at the steady state of contraction induced by 60 mM K^+ depolarization were assigned values of 0 and 100%, respectively. (b and c) Temporal changes in the $[Ca^{2+}]_i$-force relation during contraction induced by 100 nM LTC_4 (b) and 10 μM carbachol (c), using the data points 0, 5, 10, 20, 60 s after application shown in Figure 2b, c for LTC_4 and Figure 2e, f for carbachol. The solid symbols indicate the $[Ca^{2+}]_i$-force relationship obtained by the eight similar experiments shown in (a). The curved arrows show the temporal change of the $[Ca^{2+}]_i$-force relationship induced by 100 nM LTC_4 and 10 μM carbachol.

plus 10 μM SK&F 96365, the maintained increases in $[Ca^{2+}]_i$ and force induced by LTC_4 or carbachol could be completely inhibited, although the initial transient increases in $[Ca^{2+}]_i$ and force could be observed in the case of carbachol stimulation (Figure 6c, d). Figure 6e, f show a summary of the effect of diltiazem, SK&F 96365 and diltiazem plus SK&F 96365 on the $[Ca^{2+}]_i$ and force induced by LTC_4 or carbachol at a time point of 15 min after stimulation. As a result, no significant difference was observed in the pattern of inhibition by these drugs between LTC_4 and carbachol stimulation.

Discussion

The major findings in the present study are as follows: (1) Both LTC_4 and carbachol induced increases in $[Ca^{2+}]_i$ and force in the guinea-pig taenia coli. (2) Carbachol induced a large increase in Ca^{2+} sensitivity of the contractile apparatus, while LTC_4 had little or no effect on Ca^{2+} sensitivity. (3) The carbachol-induced increase in $[Ca^{2+}]_i$ was caused by both intracellular Ca^{2+} release and Ca^{2+} influx, while LTC_4-induced increase in $[Ca^{2+}]_i$ was mainly caused by Ca^{2+} influx from the extracellular space. (4) LTC_4 induces the contrac-

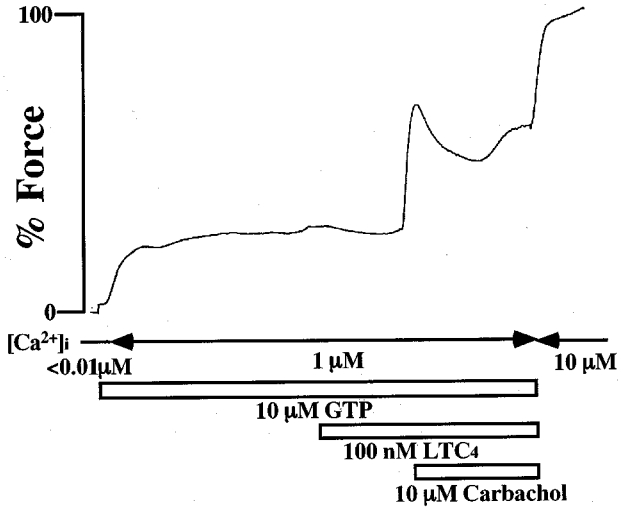

Figure 4 Effect of LTC_4 and carbachol on the Ca^{2+}-induced contraction of the α-toxin permeabilized guinea-pig taenia coli. The application of 100 nM LTC_4 and 10 μM carbachol during the maintained contraction induced by a 1 μM Ca^{2+} solution containing 10 μM GTP induced only a small increase in force at a constant $[Ca^{2+}]_i$. The application of 10 μM carbachol induced a large increase in force at a constant $[Ca^{2+}]_i$ under the same conditions.

tion of the guinea-pig taenia coli mainly through Ca^{2+} influx *via* both diltiazem-sensitive and SK&F 96365-sensitive Ca^{2+} channels. These results indicated that the mechanism underlying the LTC_4-induced contraction is greatly different from that for conventional G-protein mediated agonists, such as carbachol.

As shown in Figure 1, LTC_4 induced a concentration-dependent contraction of the guinea-pig taenia coli. Because LTC_4 induced a contraction at less than a 1 nM concentration, LTC_4 is thus suggested to be a very potent and specific contractile agonist of the guinea-pig taenia coli. These results were agreed closely with those from a previous study reported by Zschauer *et al.* (1988). Concerning the mechanism for the contraction induced by LTC_4, these authors concluded that LTC_4-induced contraction was solely dependent on the Ca^{2+} influx from the extracellular space, based on the observation that LTC_4 could not induce any tension development in the absence of extracellular Ca^{2+}. If this is the case, it is clear that the mechanism for LTC_4-induced contraction could thus be fundamentally different from that for the conventional G-protein coupled agonists, since recent research on smooth muscle contraction indicated that the these agonists induce intracellular Ca^{2+} release, Ca^{2+} influx from the extracellular space and increase in the Ca^{2+} sensitivity of the contractile apparatus (Somlyo & Somlyo, 1994; 1999). The present study was designed to determine the mechanism underlying the effect of LTC_4 on the intracellular Ca^{2+} release, on the Ca^{2+} influx and on the Ca^{2+} sensitivity.

Both LTC_4 and carbachol induced increases in $[Ca^{2+}]_i$ and force in the guinea-pig taenia coli (Figure 2). However, the maximum force induced by 100 nM LTC_4 was significantly smaller than that induced by 10 μM carbachol, although the rise in $[Ca^{2+}]_i$ was similar for both. In addition, the relationship between $[Ca^{2+}]_i$ and force during the activation by LTC_4 was similar to that during activation by 60 mM K^+-PSS, which is expected to induce no increase in Ca^{2+} sensitivity. It is thus speculated that carbachol, but not LTC_4 nor 60 mM K^+-PSS, induces an increase in the Ca^{2+} sensitivity of the contractile apparatus. This speculation was further confirmed by the experiments using intact (Figure 3) and permeabilized (Figure 4) preparations. From these results, we conclude that LTC_4 has little or no effect on the Ca^{2+} sensitivity.

Since the LTC_4-induced contraction was not accompanied by an increase in Ca^{2+} sensitivity, the LTC_4-induced contraction is solely dependent on the increase in $[Ca^{2+}]_i$. We next examined the effect of LTC_4 and carbachol on the intracellular Ca^{2+} release. As shown in Figure 5, LTC_4 did not induce a significant increase in $[Ca^{2+}]_i$ and force in the absence of the extracellular Ca^{2+}, thus indicating that LTC_4 has little or no effect on the intracellular Ca^{2+} release. On the other hand, carbachol induced the intracellular Ca^{2+} release under the same conditions as LTC_4. Carbachol has been shown to activate G protein through the stimulation of the muscarinic acetylcholine receptor and thus generate inositol 1,4,5-trisphosphate (IP_3), which causes Ca^{2+} release from the sarcoplasmic reticulum (Bolton & Lim, 1989; Komori *et al.*, 1995). It is thus speculated that LTC_4 might activate the receptor signal transduction pathways, which are different from those coupled with muscarinic and/or other classical agonists receptors. In addition, the slower rate of increase in $[Ca^{2+}]_i$ and force upon the addition of LTC_4 (Figure 2), compared with that upon the addition of carbachol, may be explained by the absence of the intracellular Ca^{2+} release mechanism.

The LTC_4-induced contraction was not accompanied by a increase in Ca^{2+} sensitivity nor the intracellular Ca^{2+} release. It follows that the LTC_4-induced contraction is solely dependent on the Ca^{2+} influx from the extracellular space, which is the same conclusion as that made by Zschauer *et al.* (1988), but which they came to using different methods. We further explored the Ca^{2+} influx pathways using two different types of Ca^{2+} channel blockers, diltiazem and SK&F 96365 (Merritt *et al.*, 1990; Zholos *et al.*, 2000). The Ca^{2+} influx pathway during activation by LTC_4 and carbachol was thought to be both the diltiazem-sensitive and SK&F 96365-sensitive Ca^{2+} channel, since the maintained increase in $[Ca^{2+}]_i$ was almost completely abolished by the pretreatment of the strips with 10 μM diltiazem plus 10 μM SK&F 96365 (Figure 6). These findings could not be accounted for by the involvement of the single type of Ca^{2+} channel in the LTC_4-induced Ca^{2+} influx. Recently, the capacitative Ca^{2+} influx pathway has been shown to play an important role in the agonist-induced increase in $[Ca^{2+}]_i$ (Berridge, 1995; Smaili *et al.*, 1998). However, this mechanism may not be activated during the stimulation with LTC_4, because LTC_4 does not induce Ca^{2+} store depletion. In addition, the transient increase in $[Ca^{2+}]_i$ induced by carbachol after the treatment with 10 μM diltiazem plus 10 μM SK&F 96365 could be explained by the intracellular Ca^{2+} release mechanisms, because LTC_4, which does not induce the intracellular Ca^{2+} release as discussed above, did not induce a transient increase in $[Ca^{2+}]_i$ under the same conditions.

In summary, the mechanism for the LTC_4-induced contraction was fundamentally different from that for the

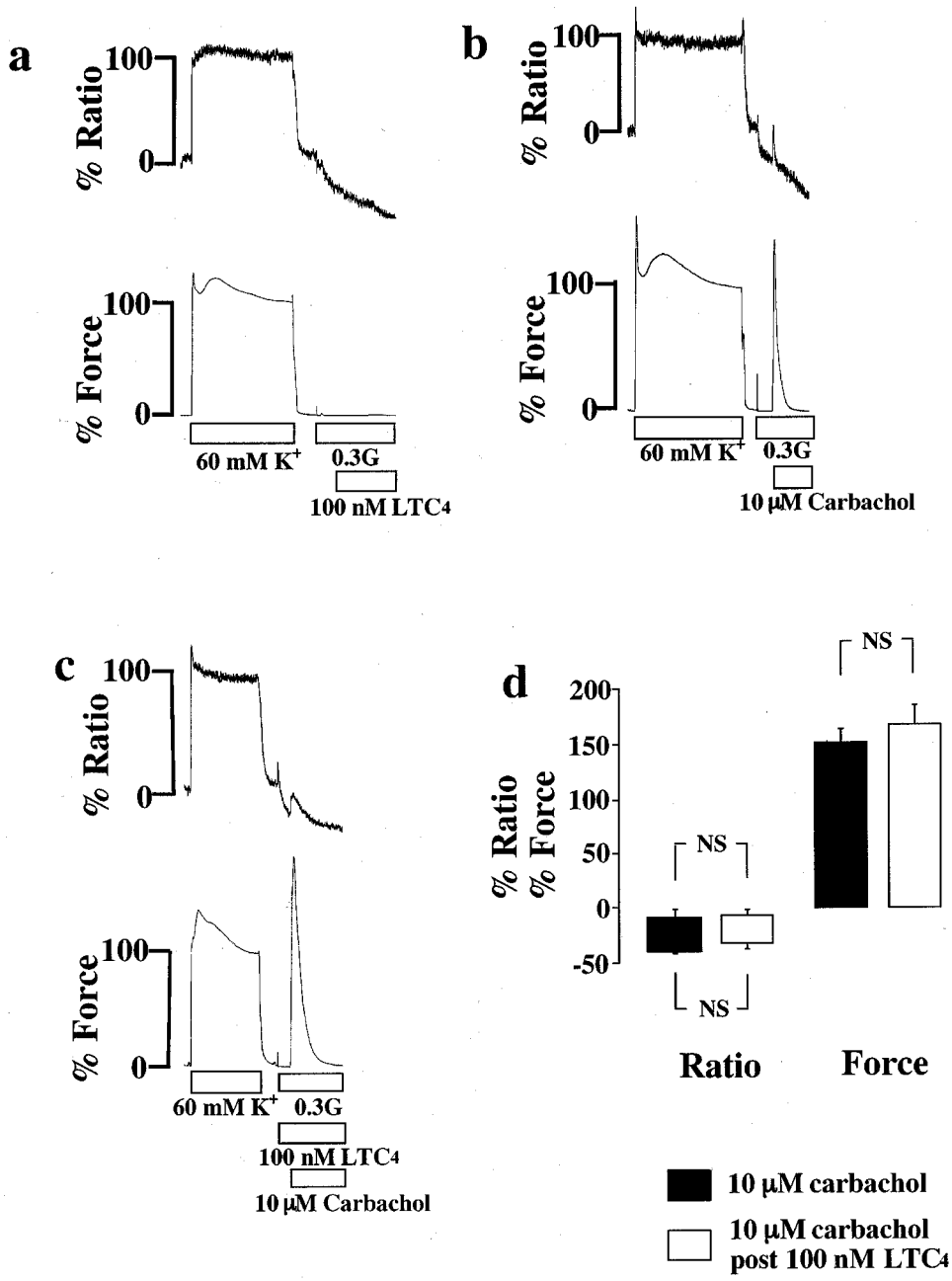

Figure 5 Effect of LTC_4 and carbachol on the $[Ca^{2+}]_i$ and force in the Ca^{2+}-free PSS containing EGTA. (a) A representative recording showing the effect of 100 nM LTC_4 on the $[Ca^{2+}]_i$ and force of the guinea-pig taenia coli in the Ca^{2+}-free PSS containing 0.3 mM EGTA. LTC_4 induced no increases in the $[Ca^{2+}]_i$ and force. (b) A representative recording showing the effect of 10 μM carbachol on the $[Ca^{2+}]_i$ and force in the Ca^{2+}-free PSS containing 0.3 mM EGTA. Carbachol induced transient increases in the $[Ca^{2+}]_i$ and force. (c) A representative recording showing the effect of 10 μM carbachol on the $[Ca^{2+}]_i$ and force in the Ca^{2+}-free PSS containing 0.3 mM EGTA and 100 nM LTC_4. Carbachol induced transient increases in the $[Ca^{2+}]_i$ and force. (d) A summary of the effects of 10 μM carbachol on the $[Ca^{2+}]_i$ and force of guinea-pig taenia coli in the Ca^{2+}-free PSS containing 0.3 mM EGTA with or without 100 nM LTC_4. The $[Ca^{2+}]_i$ and developed force were expressed as a percentage, assigning the value in normal (5.9 mM K^+) PSS and 60 mM K^+ PSS to be 0 and 100%, respectively. Data are the mean$\pm$s.e.mean ($n=6-8$). The bottom line of each column represents the level of $[Ca^{2+}]_i$ and force before stimulation with carbachol. The top line of each column represents the maximum levels of $[Ca^{2+}]_i$ and force after stimulation.

carbachol-induced contraction. Carbachol induces intracellular Ca^{2+} release, Ca^{2+} influx and increase in Ca^{2+} sensitivity, while LTC_4 induces Ca^{2+} influx alone. These differences may be explained by differences in the receptor signal transduction system coupled with each receptor, although the precise mechanism for this is unknown at present, due primarily to the fact that LTC_4 receptors have not yet been identified or cloned.

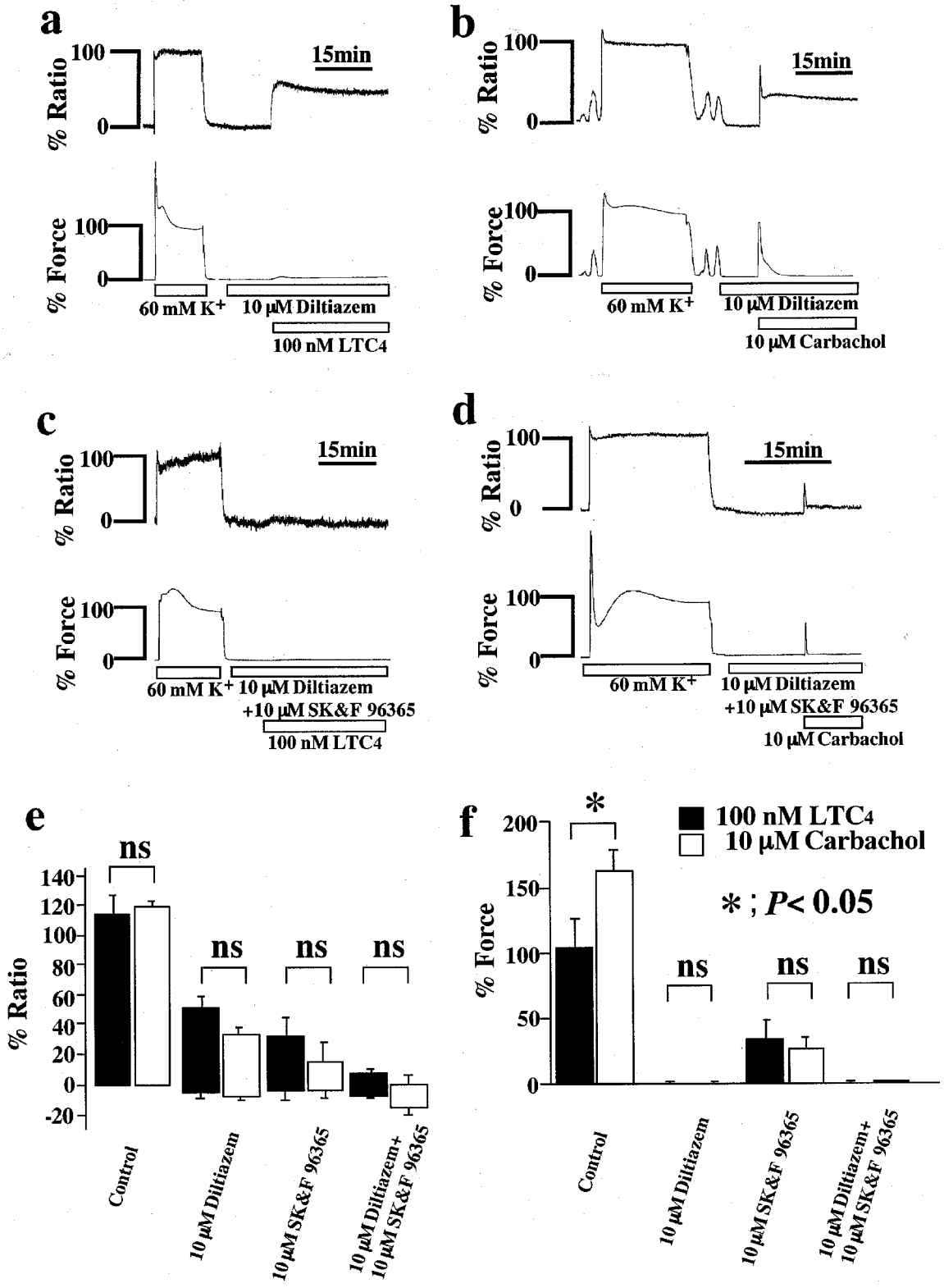

Figure 6 Effects of diltiazem and SK&F 96365 on the LTC_4- and carbachol-induced changes in $[Ca^{2+}]_i$ and force in guinea-pig taenia coli. (a) A representative recording showing the effect of 10 μM diltiazem on the 100 nM LTC_4-induced increases in $[Ca^{2+}]_i$ and force. Diltiazem was applied 10 min before and during LTC_4 administration. (b) A representative recording showing the effect of 10 μM diltiazem on the 10 μM carbachol-induced increases in $[Ca^{2+}]_i$ and force. (c) Effect of 10 μM diltiazem plus 10 μM SK&F 96365 on the 100 nM LTC_4-induced increases in $[Ca^{2+}]_i$ and force. (d) Effect of 10 μM diltiazem plus 10 μM SK&F 96365 on the 10 μM carbachol-induced increases in $[Ca^{2+}]_i$ and force. (e and f) A summary of the inhibitory effect of diltiazem and SK&F 96365 on the LTC_4 and carbachol induced increases in $[Ca^{2+}]_i$ (e) and force (f). Data are the means $\pm$ s.e.mean ($n = 5-8$).

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