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The roles of stored calcium in contractions of cat tracheal smooth muscle produced by electrical stimulation, acetylcholine and high K^+

Yushi Ito & Takeo Itoh

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

1 Effects of direct or indirect (nerve-mediated) muscle stimulation, acetylcholine (ACh), caffeine and procaine on the membrane and mechanical properties of smooth muscle cells of the cat trachea were investigated by means of double sucrose-gap and isometric tension recording methods.

2 Outward current pulses (2 s in duration) applied to the muscle tissue in the presence of tetrodotoxin (10^{-7}M), atropine (10^{-6}M) and propranolol (10^{-6}M) evoked no action potential (spike); however, when the depolarization exceeded 9 mV, a contraction was evoked.

3 The spike and contraction evoked by outward current pulses in the presence of tetraethylammonium (TEA, 10 mM) were suppressed by treatment of the tissue with either Ca^{2+}-free EGTA (2 mM) containing solution or Mn^{2+} (5 mM). In the presence of procaine (10 mM), outward current pulses evoked an action potential but no contraction.

4 Field stimulation of short duration (50 μs) applied to the whole tissue produced an excitation of the intrinsic nerves and evoked excitatory junction potentials (e.j.ps), and when the amplitude of e.j.ps exceeded 4 mV, a twitch contraction occurred. E.j.p. was more effective in producing a contraction than was the membrane depolarization evoked by outward current pulses.

5 Amplitudes of contractions evoked by exogenous ACh (10^{-5}M) were much larger than those evoked by 128 mM-$[K]_0$ or caffeine (10 mM), in normal Krebs solution. When the amplitudes of the contractions produced by 128 mM $[K]_0$ were defined as a relative amplitude of 1.0, the mean amplitudes of contraction produced by ACh (10^{-5}M) or caffeine were 2.5 ± 0.20 or 1.2 ± 0.26, respectively.

6 In Ca^{2+}-free EGTA (2 mM)-containing solution, the contraction induced by 128 mM-$[K]_0$ was rapidly abolished, whereas the contractions evoked by caffeine (10 mM) or the initial phasic contraction produced by ACh (10^{-5}M) were largely unaffected.

7 When the amount of Ca^{2+} stored in the muscle cell was estimated from the amplitude of caffeine-induced contraction evoked in Ca^{2+}-free solution, procaine (10 mM) applied simultaneously with Ca^{2+}, after depletion of Ca^{2+} from the cells by means of caffeine, increased the amount of Ca^{2+} stored to 1.31 ± 0.14 ($n = 6$) times the control value. However, ACh (10^{-7}M) or excess concentrations of $[K]_0$ applied with Ca^{2+} did not increase the amount of Ca^{2+} stored in the caffeine-sensitive intracellular compartment.

8 These results indicate that the amount of Ca^{2+} stored in the smooth muscle cells of the cat trachea may be larger than other visceral smooth muscle and plays an important role in the initiation of contraction, in response to endogenous or exogenous ACh.

Introduction

In smooth muscle cells, the contraction-relaxation cycle is largely dependent on the regulation of ionized free calcium in the cytoplasm, as in the case of skeletal or cardiac muscles (see for example Kuriyama *et al.*, 1982), and the sources of Ca^{2+} contributing to the activation of contractile proteins are of extra- and intracellular origins.

Itoh *et al.* (1981) concluded that in case of the guinea-pig mesenteric artery, the Ca^{2+} inward current generated at the myoplasmic membrane may not directly provide the free Ca^{2+} required to activate the contractile proteins, but that the Ca^{2+} carrying the inward current may cause the release of intracellular Ca^{2+} (Ca^{2+}-induced Ca^{2+} release) which in

turn leads to contraction. However, it remains to be clarified whether or not this mechanism is the main process involved in induction of contractions in various smooth muscle cells, since species and organ specificities are prominent in these cells (see for example Kuriyama, 1981).

Respiratory airways contain smooth muscle cells which have both similar and distinct electrophysiological properties from those of vascular smooth muscles. For example, most of the smooth muscle cells in vascular or airway tissues have little spontaneous mechanical activity and are normally electrically quiescent. Application of outward current pulses to the smooth muscle cells does not evoke an action potential in normal Krebs solution. However, in the guinea-pig mesenteric artery, the excitatory junction potential (e.j.p.) evoked by perivascular nerve stimulation, which is due to the release of noradrenaline from the sympathetic nerve terminal, elicits an action potential followed by twitch contraction of the muscle cell (Suzuki & Kuriyama, 1980; Itoh *et al.*, 1981). On the other hand, in the tracheal smooth muscle, although e.j.ps, which are due to the release of acetylcholine from the parasympathetic nerve terminals, do not trigger an action potential, they evoke twitch contractions of the muscle cells (Kirkpatrick, 1981; Ito & Tajima, 1981; Ito & Takeda, 1982). Thus, direct or indirect muscle stimulation does not evoke an action potential in the tracheal smooth muscle cells.

We investigated the sources of Ca^{2+} which activate the contractile protein in the airway smooth muscle in response to direct and indirect electrical stimulation and to various agents, and comparisons were made with findings in other visceral smooth muscles.

For these experiments, we used the cat tracheal tissues, since electrical membrane properties of the smooth muscle and neuro-effector transmission have already been studied in detail in this species (Ito & Takeda, 1982).

Methods

Adult cats of either sex weighing 2 – 3 kg were anaesthetized with pentobarbitone (30 mg^{-1} kg, i.v.) and exsanguinated. Segments of the cervical trachea were excised, and a dorsal strip of transversely running smooth muscle fibre was separated from the cartilage. The mucosa and adventitial tissue were carefully removed, under microscopic observation.

The preparation was bathed in a modified Krebs solution (hereafter referred to as Krebs solution) with the following ionic concentration (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5 The Ca^{2+}-free EGTA (2 mM)-containing solution was prepared by replacing 2.5 mM $CaCl_2$ with equimolar $MgCl_2$ and adding 2 mM EGTA. The solution was aerated with 97% O_2 and 3% CO_2 and the pH was adjusted to 7.3 – 7.4.

The double sucrose-gap method was used to record the membrane potential and tension development in the tissue. The chamber used was as described elsewhere (Ito & Tajima, 1981). A single stimulation was applied using a current pulse of 50 μs in duration and 10 – 30 V in strength to stimulate intrinsic nerves of the tissue. To stimulate the smooth muscle cells, current pulses of 2 s in duration and 1 – 5 V in strength were applied in the presence of tetrodotoxin (10^{-7}M), atropine (10^{-6}M) and propranolol (10^{-6}M).

For tension recording, the muscle strips were cut to a width of 0.05 – 0.1 mm and a length of about 0.3 mm, under a binocular microscope. The preparation was set up in a small chamber with a capacity of 0.9 ml through which the test solution was superfused rapidly (Itoh *et al.*, 1981). Both ends of the preparation were fixed between pieces of Scotch double tape (3M Co., St. Paul, MN), and isometric tension was recorded with a strain gauge transducer (U-gauge, Shinko Co.).

The amount of Ca^{2+} stored in the muscle cell was estimated from the amplitude of caffeine-induced contraction. Firstly, the tissue was treated with Ca^{2+}-free EGTA-containing solution for 10 min during which time caffeine (10 mM) was applied once to deplete stored Ca^{2+}. Secondly, the tissue was loaded with 2.5 mM Ca^{2+} for 5 min and then caffeine (10 mM) was applied to estimate the amount of stored Ca^{2+} from the caffeine-induced contraction after 4 min treatment with Ca^{2+}-free EGTA containing solution. Thus, the caffeine-sensitive intracellular Ca^{2+} store will be referred to in the text as the 'Ca^{2+} store' or 'stored Ca'.

The following drugs were used: acetylcholine Cl (SIGMA), caffeine (Wako), procaine HCl (SIGMA), ethyleneglycol-*bis* (β-aminoethylether)-N- N'- tetra acetic acid (EGTA; Dozin) and atropine sulphate (Merck), propranolol (Sumitomo) and tetraethylammonium Cl (TEA; SIGMA).

Results (amplitude of e.j.ps and contractions) were expressed as means ± s.d, and analysed for significance by Student's *t* test.

Results

Effects of tetraethylammonium and procaine on the excitation-contraction coupling of the cat tracheal muscle

Outward current pulses (2 s duration) applied to the muscle cells did not produce an action potential in the

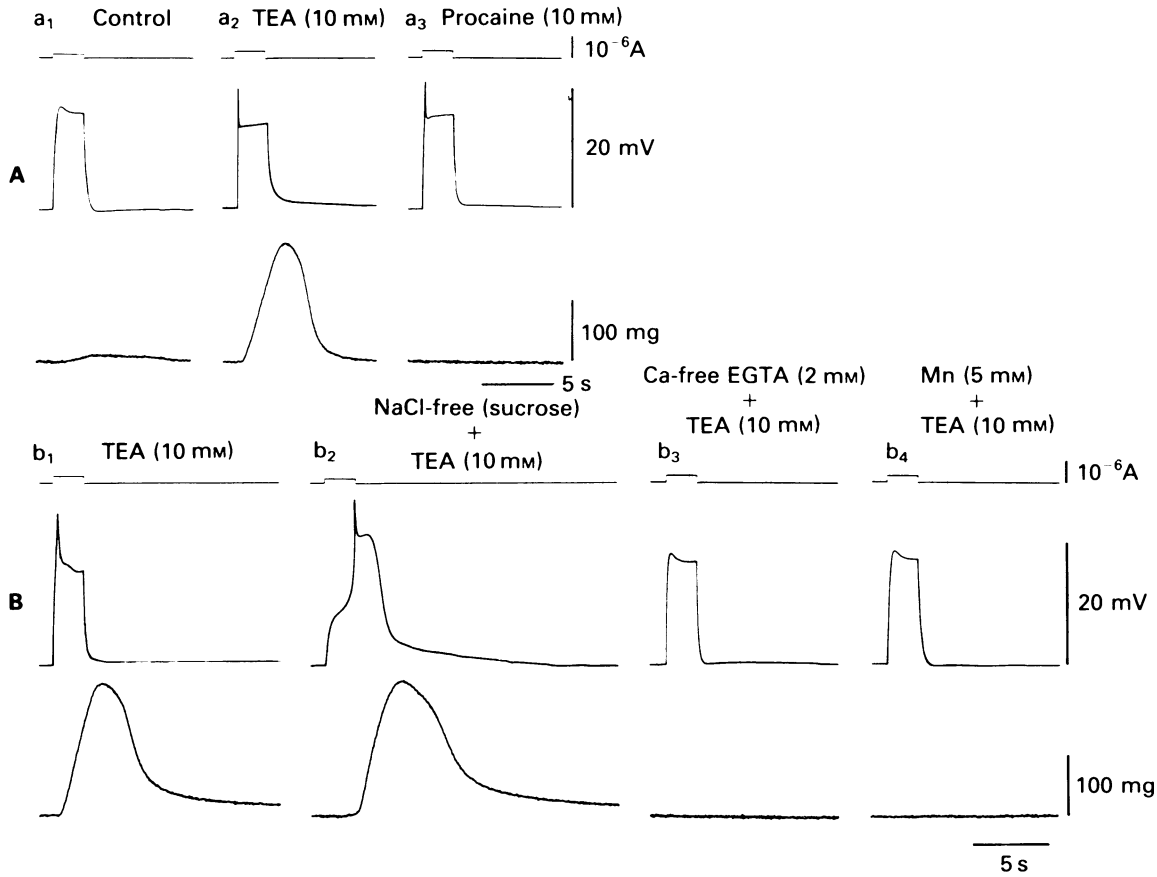

Figure 1 Electrical and mechanical responses of smooth muscle cells of the cat trachea evoked by outward current pulses (2 s duration, 1 – 5 V) in the presence of tetrodotoxin (TTX 10^{-7} M), atropine (10^{-6} M) and propranolol (10^{-6} M). (A) Effects of TEA (10 mM; a_2) or procaine (10 mM; a_3) and (B) effects of NaCl-free (b_2), Ca^{2+}-free EGTA-containing solution (b_3) or 5 mM Mn^{2+} (b_4) on the action potential and tension development in the presence of TEA (10 mM).

presence of tetrodotoxin (10^{-7}M), atropine (10^{-6}M) and propranolol (10^{-6}M). However, after treatment of the tissue with TEA (10 mM), outward current pulses produced a spike and a large contraction (about 10 times larger than the contraction evoked by 30 mV membrane depolarization produced by outward current pulse in Krebs solution), when the depolarization exceeded 10 mV (Figure 1 A). To study the ionic mechanisms involved in the generation of action potential in the presence of TEA, effects of Na^+, Ca^{2+} or Mn^{2+} on the action potential were observed. When Na^+-free or NaCl-free solution was prepared by substitution of Na^+ with Tris Cl or sucrose, the action potential was generated and a contraction was produced in response to muscle stimulation (Figure 1 b_2). However, in the presence of Ca^{2+}-free EGTA-containing Krebs or Mn^{2+} (5.0 mM) solution, action potential generation and contraction were abolished. These results indicate that the action potential evoked in the presence of TEA in these smooth muscle cells was due to activation of Ca^{2+} influx, which in turn induced contraction.

Figure 1 A also shows the effects of procaine (10 mM) on the electrical and mechanical properties of the smooth muscle cells. Procaine (10 mM) slightly depolarized the membrane (2 – 3 mV), as measured by the double sucrose gap method, and generation of action potential was consistently observed in response to the outward current pulses. However, contraction ceased, i.e. decoupling of the excitation-contraction process occurred. This means that Ca ions entering the cell during the action potential do not directly activate the contractile machinery.

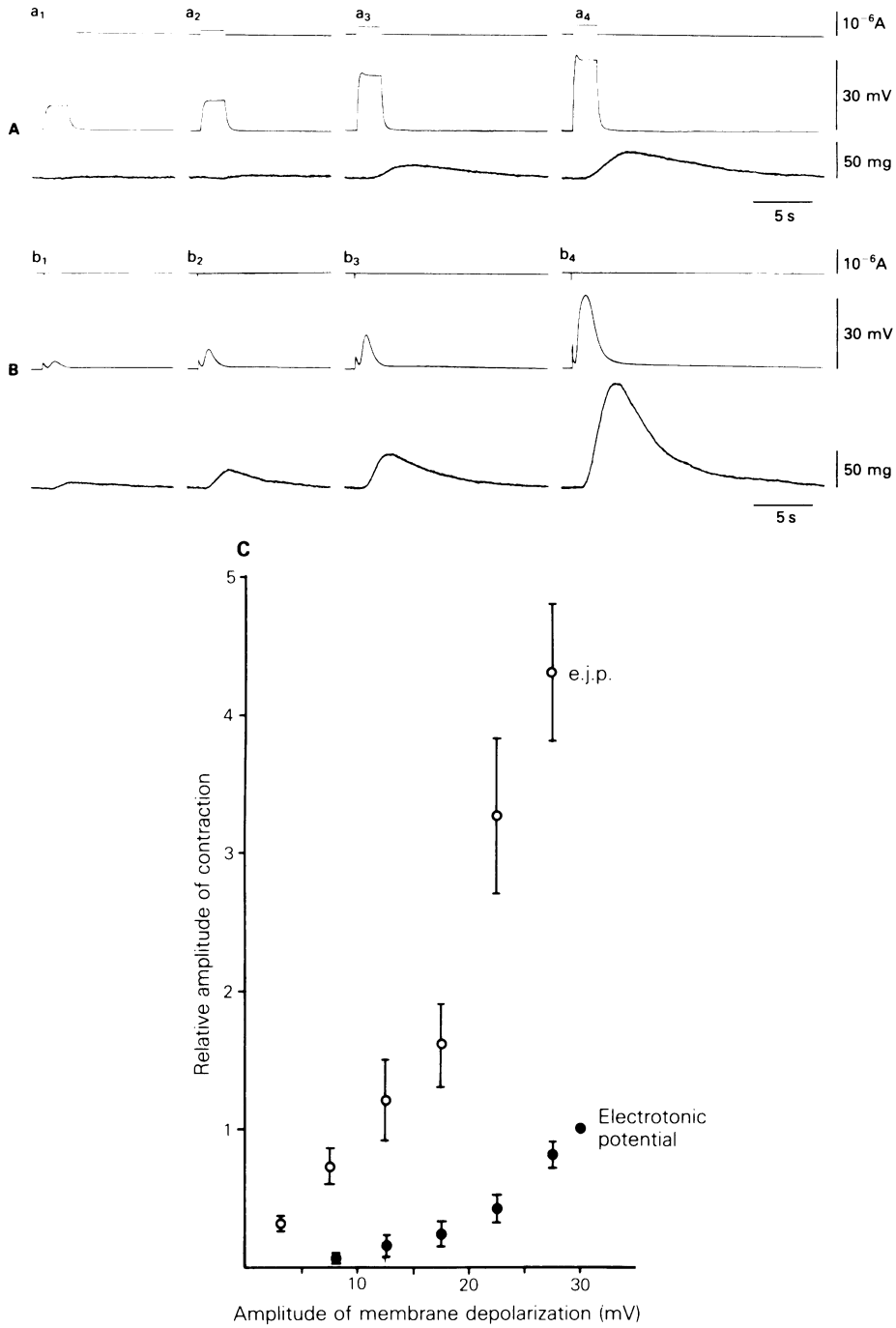

Figure 2 Depolarization-contraction relationship observed with electrotonic potential evoked by outward current pulses (2 s duration, $2-4\times10^{-7}$ A) (A) or e.j.ps (50 μs duration $0.5-2\times10^{-7}$ A) (B). (C) Relationship between the amplitude of membrane depolarization and relative amplitude of contractions. The amplitude of contraction evoked by 30 mV depolarization (electrotonic potential) was normalized as 1.0. Each point indicates the mean value of five to six experiments, and vertical bars indicate 2 × s.d.

Effects of direct and indirect muscle stimulation on the contractile response of the cat trachea

Figure 2 shows the electrical and mechanical responses of the cat tracheal smooth muscle to direct or indirect (nerve-mediated) muscle stimulation observed using the double sucrose-gap method.

Outward current pulses (2 s duration) applied to the muscle cells evoked contractions in the presence of tetrodotoxin (10^{-7}M), atropine (10^{-6}M) and propranolol (10^{-6}M), when the membrane depolarization exceeded 9 mV (Figure 2 A). The amplitude of the contraction increased in proportion to the amplitude of membrane depolarization (Figure 2 A),

Single field stimulation of short duration (50 μs) evoked an excitatory junction potential (e.j.p.) followed by a twitch contraction in the presence of propranolol (10^{-6}M). To observe the relationship between the amplitude of e.j.ps and contractions, field stimulations of various intensities and at a con-

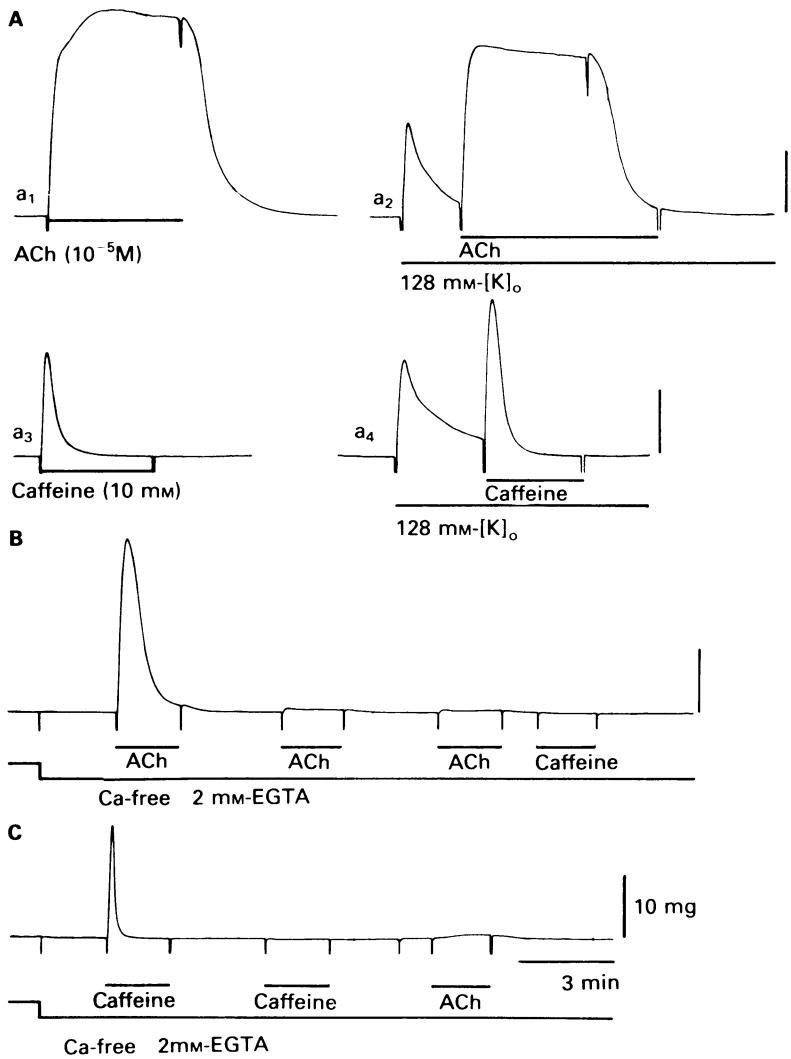

Figure 3 (A) Effects of acetylcholine (ACh 10^{-5}M), caffeine (10 mM) or 128 mM-$[K]_0$ on the mechanical properties of the cat trachea in the normal Krebs solution. In a_2 or a_4, ACh (10^{-5}M) or caffeine (10 mM) were applied during tonic contractures evoked by 128 mM-$[K]_0$, respectively. (B and C) Effects of Ca^{2+}-free 2 mM-EGTA containing solution on ACh- or caffeine-induced contractions. ACh (10^{-5}M) or caffeine (10 mM) were applied 2 min after application of Ca^{2+}-free EGTA containing solution. In (B) caffeine (10 mM) was applied following three applications of ACh (10^{-5}M) and in (C) ACh (10^{-5}M) was applied after repetitive applications of caffeine (10 mM).

stant pulse duration (50 μs) were applied to the tissue. The minimum membrane depolarization of e.j.p. required to evoke a contraction was in the range between 3 to 5 mV (mean 4.5 ± 1.5 mV, $n = 5$), and the amplitude of twitch contraction increased in proportion to the amplitude of the e.j.p. (Figure 2 B). Figure 2 C shows the relationship between the amplitude of membrane depolarization evoked by outward current pulses (2 s in duration) or e.j.p. and the relative amplitude of contractions. The membrane depolarization evoked by e.j.p. was more effective in inducing tension development than that induced by the outward current pulses.

Effects of acetylcholine, caffeine, or excess-$[K]_o$ on the contractile properties of the cat trachea

The sources of Ca^{2+} involved in contractions evoked by ACh, caffeine or excess-$[K]_o$ were investigated.

Figure 3 A shows contractions evoked by ACh (10^{-5} M), 128 mM-$[K]_o$ or caffeine (10 mM). The maximum amplitudes of the ACh-induced contractions were much larger than those evoked by 128 mM-$[K]_o$ or caffeine (10 mM). When the amplitude of phasic contraction evoked by 128 mM-$[K]_o$ was registered as a relative amplitude of 1.0, the mean amplitudes of maximum contraction evoked by ACh (10^{-5} M) or caffeine (10 mM) were 2.5 ± 0.20 ($n = 10$) or 1.2 ± 0.26 ($n = 9$), respectively. Furthermore, application of ACh (10^{-5} M) or caffeine (10 mM), during the tonic contraction evoked by 128 mM-$[K]_o$, evoked contractions with a larger amplitude than those evoked by 128 mM-$[K]_o$. This indicates that the sources of the Ca^{2+} which activate the contractile protein differ in the case of excess-$[K]_o$ and ACh or caffeine (Figure 3 A).

ACh-induced contraction in Krebs solution was biphasic, i.e. an initial phasic response followed by a sustained tonic response. When Ca^{2+}-free EGTA-containing solution was applied during sustained contraction evoked by ACh (10^{-5} M), the tissue rapidly relaxed close to the resting level (data not shown). Furthermore, after treatment with Ca^{2+}-free EGTA-containing solution for 2 min, ACh (10^{-5} M) evoked the phasic response only, i.e. in Ca^{2+}-free EGTA-containing solution the initial phasic component persisted with slight reduction in the amplitude (10% decrease), but the tonic component of ACh-induced contraction was abolished. During repetitive application of ACh (10^{-5} M) in Ca^{2+}-free EGTA-containing solution, the amplitude of the phasic response was reduced to less than 5% of the initial value. However, when caffeine (10 mM) was applied following three applications of ACh, a mechanical response was not evoked. Similar to the phasic components of ACh-induced contraction, caffeine (10 mM) also evoked phasic contractions in Ca^{2+}-free EGTA-containing solution. However, the contraction was not evoked upon the second application of caffeine in the presence of Ca^{2+}-free EGTA-containing solution, although application of ACh (after 12 min superfusion with Ca^{2+}-free EGTA-containing solution) produced minute contractions (Figure 3C).

The effects of Ca^{2+}-free solution on excess concentrations of $[K]_o$-induced contractions were observed. Tissues were pretreated with Ca^{2+}-free EGTA-containing solution for 3 min and various concentrations of excess $[K]_o$ were applied which did

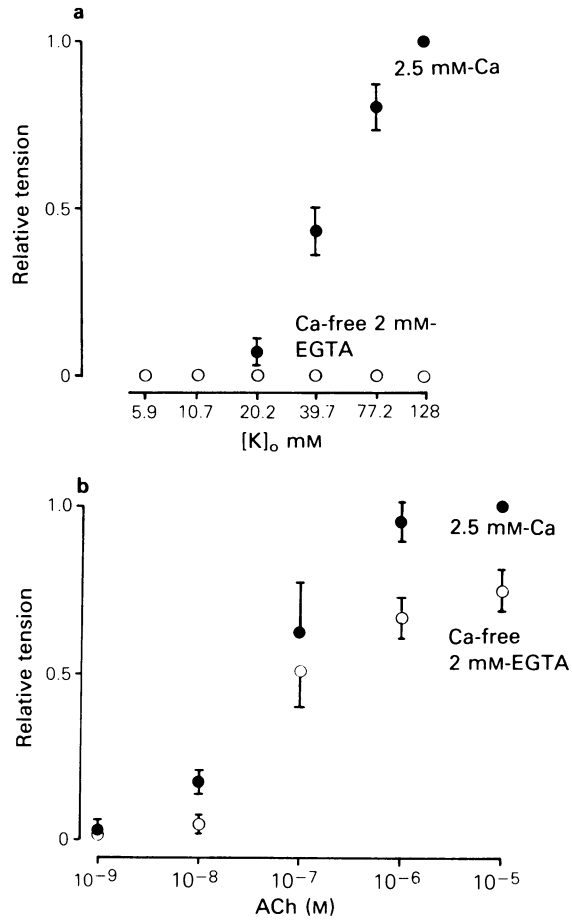

Figure 4 Effects of incubation in Ca^{2+}-free 2 mM EGTA-containing Krebs for 3 min on the contractions to excess $[K]_o$ (A) or the initial phasic contraction evoked by acetylcholine (ACh) (b). (●) Control; (○) in Ca^{2+}-free 2 mM EGTA-containing solution. Responses are expressed as a relative amplitude of the maximum contraction, evoked by 128 mM $[K]_o$ or 10^{-5} M ACh, respectively. Each point indicates the mean value of four to six experiments, and vertical bars indicate 2 × s.d.

not evoke a contraction in Ca^{2+}-free EGTA-containing solution (Figure 4a).

Figure 4b shows the relationship between the concentration of ACh and the relative tension observed in normal or in Ca^{2+}-free EGTA-containing solution. When the tissue was treated with Ca^{2+}-free EGTA-containing solution for 3 min the ACh (10^{-8} – 10^{-5}M)-induced contractions were evoked with a reduction in the amplitude (to 30 – 80% of the control value).

These results indicate that the K^+-induced contraction is mainly due to influx of Ca^{2+}, while initial phasic contraction produced by ACh or caffeine are mainly due to release of Ca^{2+} stored in the cells. A major part of the ACh-sensitive Ca^{2+} store site may be the same as that of caffeine.

Effects of Ca^{2+} loading or Ca^{2+}-free EGTA containing solution on the amount of Ca^{2+} stored in the muscle tissue

To assess the properties of the Ca^{2+}-storage sites in smooth muscle cells of the cat trachea, the following experiments were done. The tissue was treated with Ca^{2+}-free EGTA-containing solution for 10 min, during which time caffeine (10 mM) was applied once to deplete the stored Ca^{2+}. Ca^{2+} 2.5 mM was then applied for various times and after 2 min incubation in Ca^{2+}-free solution caffeine (10 mM) was applied in order to estimate the amount of stored Ca^{2+} from the amplitude of caffeine-induced contractions. The amplitude of caffeine-induced contraction increased in parallel with the duration of incubation time in 2.5 mM Ca^{2+} Krebs and reached a maximum after 10 min (Figure 5a). These results indicate that after depletion of the stored Ca^{2+}, application of 2.5 mM Ca^{2+} for about 10 min replenishes Ca^{2+} in the storage sites. Figure 5b shows the effects of duration of superfusion with Ca^{2+}-free EGTA-containing solution, (after loading the tissue with 2.5 mM Ca^{2+} for 10 min), on the amplitude of caffeine-induced contraction. The amplitude of the caffeine-induced contractions was decreased in proportion to the superfusion time in Ca^{2+}-free solution. However, reduction in the amplitude was slight and after 10 min superfusion with Ca^{2+}-free EGTA containing solution, the relative amplitude of the caffeine-induced contraction was 0.90 ± 0.5 ($n = 6$) of the control value.

Effects of acetylcholine (10^{-7}M), 128 mM-$[K]_0$ or procaine (10 mM) on the caffeine-induced contraction

To investigate the effects of procaine on the ACh or $[K]_0$-induced contraction, or on the stored Ca^{2+} (i.e. caffeine-sensitive intracellular Ca^{2+} store) and the

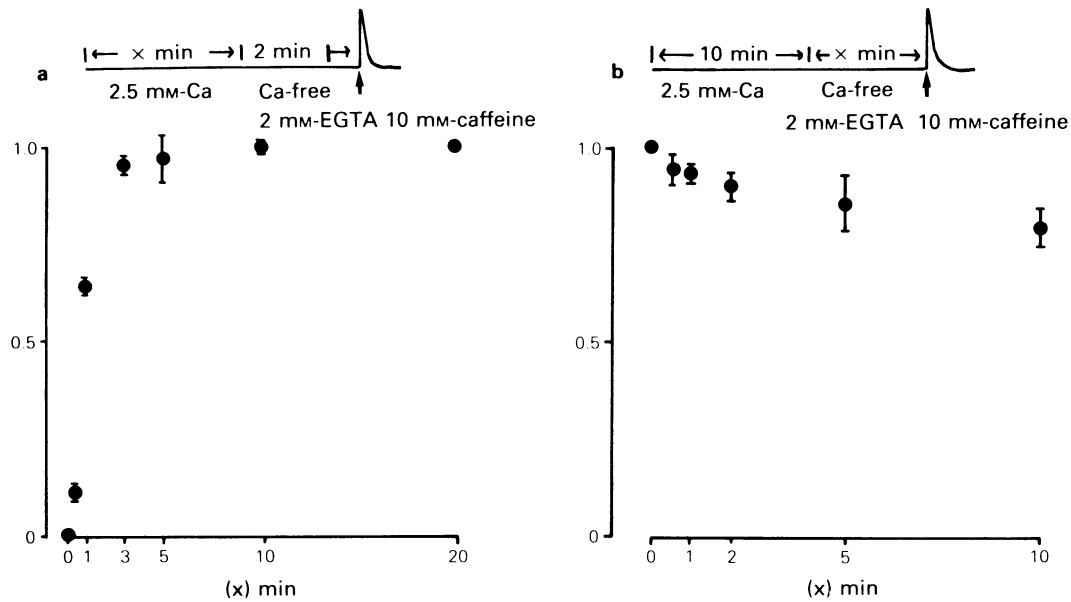

Figure 5 (a) Effects of incubation time in 2.5 mM-Ca^{2+} on the caffeine (10 mM)-induced contraction evoked in Ca^{2+}-free solution. The amplitude of caffeine-induced contraction evoked 2 min after rinsing with 2 mM-EGTA containing solution following 20 min incubation in 2.5 mM Ca^{2+} was registered as a relative tension of 1.0 (b), Effects of incubation time in Ca^{2+}-free EGTA-containing solution after loading the tissue with 2.5 mM Ca^{2+} for 10 min. The amplitude of the 10 mM caffeine-induced contraction evoked just after the loading of 2.5 mM Ca^{2+} for 10 min was registered as a relative tension of 1.0.

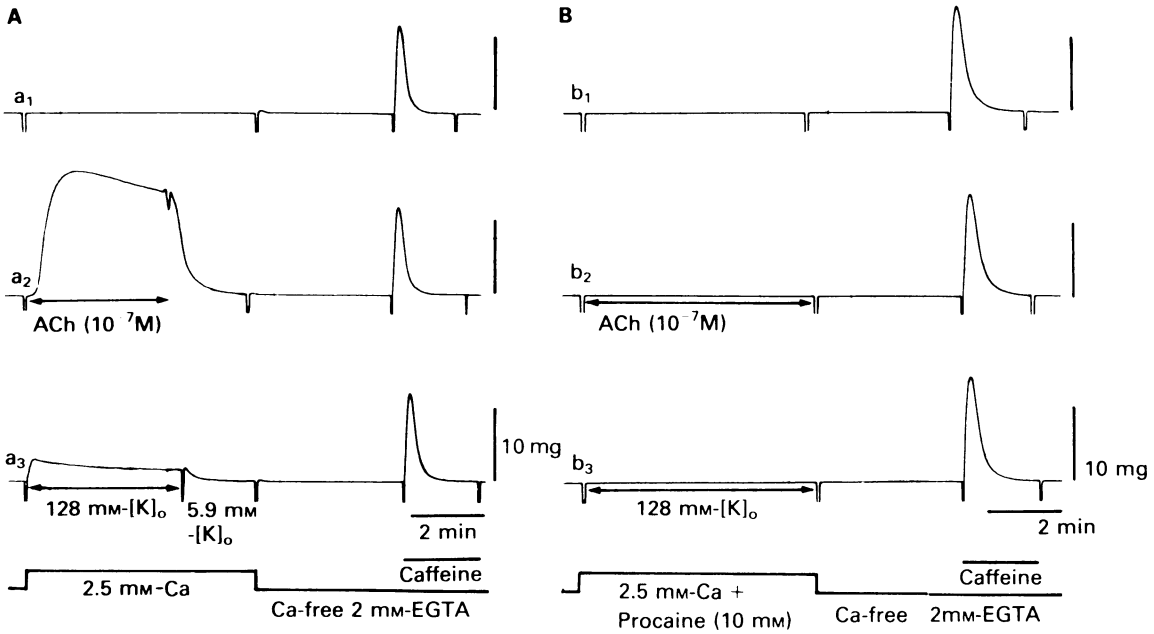

Figure 6 Effects of acetylcholine (ACh, 10^{-7}M), 128 mM-$[K]_0$ or procaine (10 mM) on the 10 mM caffeine-induced contraction in the Ca^{2+}-free 2 mM EGTA containing solution. The tissues were pretreated with Ca-free EGTA-containing solution for 10 min, and 10 mM caffeine was applied once to deplete the stored Ca. Then the tissue was loaded with 2.5 mM Ca^{2+} for 5 min. After rinsing the tissue with Ca^{2+}-free EGTA-containing solution for 4 min, caffeine was applied. ACh (10^{-7}M) or 128 mM-$[K]_0$ was applied during the loading of the tissue in the absence (A) or presence (B) of procaine (10 mM).

effects of ACh or 128 mM $[K]_0$ on the stored Ca^{2+}, the following experiments were done. After depletion of the Ca stored in cells by treatment with caffeine in Ca^{2+}-free EGTA-containing solution, the tissue was loaded with 2.5 mM Ca^{2+} for 5 min and then caffeine (10 mM) was applied to estimate the amount of stored Ca^{2+} after 4 min treatment with Ca^{2+}-free EGTA containing solution. ACh or 128 mM-$[K]_0$ were applied simultaneously with 2.5 mM Ca^{2+} in the absence or presence of procaine. As shown in Figure $6a_1$ during the application of 2.5 mM Ca^{2+} to the control tissue a contraction was not generated. However, 10 mM caffeine applied 4 min after treatment with Ca^{2+}-free solution evoked a phasic contraction. When ACh (10^{-7}M) or 128 mM-$[K]_0$ was applied simultaneously with 2.5 mM Ca^{2+}, sustained contractions occurred. However, the amplitude of the phasic contraction evoked by application of caffeine was similar to control values, indicating that ACh (10^{-7}M) or 128 mM-$[K]_0$ did not enhance the amount of Ca^{2+} accumulated in the caffeine-sensitive store sites.

On the other hand, when procaine was applied with 2.5 mM-Ca^{2+}, the amplitude of the caffeine-induced contraction was increased, dose-dependently (Figure $6b_1$ vs a_1). For example, 5 or 10 mM procaine enhanced the amplitude of caffeine-induced contraction to 1.18 ± 0.05 ($\pm$s.d., $n = 5$) or to 1.31 ± 0.14 ($\pm$s.d., $n = 6$) of the control value, respectively. Furthermore, procaine blocked the contractions evoked by ACh (10^{-7}M) or 128mM-$[K]_0$ during the application of 2.5 mM Ca^{2+}, although the amplitude of the caffeine-induced contraction was increased to the same extent as observed after incubation with 2.5 mM Ca and 10 mM procaine (Figure $6b_1 - b_3$).

Discussion

The present results show that contractile responses of the cat trachea evoked by excess concentrations of $[K]_0$ or by electrical depolarization of the membrane by outward current pulses are mainly due to Ca^{2+} influx, and that phasic contractions evoked by the application of endogenous (i.e. transmural stimulation) or exogenous ACh and caffeine are mainly due to release of Ca^{2+} from intracellular stores (mainly sarcoplasmic reticulum).

It has been reported that, in the dog trachea, the sources of Ca^{2+} responsible for ACh contractions are dependent upon the drug dose, namely at low

doses of ACh ($< 10^{-6}$M) depolarization-dependent Ca^{2+} influxes are mainly involved and at higher doses, pharmacomechanical coupling becomes more important and depolarization-dependent Ca^{2+} influx does not contribute to the contraction (Farley & Miles 1978). However, this is not the case in the cat trachea as low doses of ACh ($10^{-8} - 10^{-6}$M) evoked a phasic contraction in the presence of Ca^{2+}-free EGTA-containing solution, the amplitudes of which were in the range of 30 – 90% of the controls. Furthermore, 3 – 5 mV membrane depolarization evoked by e.j.p, but not the electrical membrane depolarization by the outward current pulses evoked a phasic contraction, indicating that e.j.p.-induced contraction is not due to membrane depolarization. Furthermore, the amplitude of twitch-like contractions evoked by e.j.p. is much larger than those evoked by membrane depolarization elicited by outward current pulses, when compared at the same level of membrane potential (5 mV – 30 mV). Although estimation of the concentration of endogenous ACh at the smooth muscle cells required to evoke e.j.ps in the range of 3 mV – 30 mV amplitude is not feasible, the present results do strongly indicate that endogenous or exogenous ($10^{-9} - 10^{-5}$M) ACh uses mainly intracellular Ca^{2+} to produce contraction.

It seems likely that in the cat trachea, the amount of intracellular Ca^{2+} in the caffeine-sensitive store is larger than in other visceral smooth muscles. A direct comparison between the present results and basal ^{45}Ca uptake studies carried out in various smooth muscles is not technically feasible, but it is possible to estimate the amount of Ca^{2+} in the caffeine-sensitive store from the amplitude of caffeine-induced contractions in Ca^{2+}-free EGTA containing solution. For example, when the amplitude of contractions evoked by 128 mM-$[K]_0$ was defined as a relative amplitude of 1.0, the amplitude of caffeine-induced contractions was 1.2 ± 0.26 ($n = 5$) in the cat trachea, and the value was only 0.63 ± 0.08 ($n = 8$) in the rabbit mesenteric artery (Itoh *et al.*, 1983). Similarly, the amplitude of the noradrenaline (10^{-5}M)-induced contractions relative to that of the 128 mM-$[K]_0$ induced contraction was only 0.68 ± 0.04 (Itoh *et al.*, 1981). It could be that the Ca^{2+} stores and the amount of Ca^{2+} released may be similar in the cat trachea and in other visceral smooth muscles, and that the observed differences in the relative size of the caffeine-induced contractions are attributable to differences in Ca^{2+} efflux and/or reuptake into intracellular locations resulting in different amounts of Ca^{2+} ultimately interacting with the contractile machinery. However, treatment of the trachealis muscles with Ca^{2+}-free EGTA-containing solution for 10 min reduced only slightly the amount of Ca^{2+} in the caffeine-sensitive store as estimated from the amplitude of the caffeine (10 mM)-induced contraction. Similar treatment of muscle specimens prepared from guinea-pig taenia coli or stomach almost depleted Ca^{2+} from the intracellular store sites (Casteels & Raeymaekers, 1979; Itoh *et al.*, 1982). All these results indicate that the amount of stored Ca^{2+} in the trachea is large and is resistant to the treatment with Ca^{2+}-free EGTA containing solution, as compared to other visceral smooth muscle cells (Itoh *et al.*, 1981; 1982; Casteels & Raeymaekers, 1979).

Furthermore, in the guinea-pig mesenteric artery, treatment with procaine (10 mM), a suppressor of the Ca^{2+}-induced Ca^{2+} release mechanisms from the storage sites in the skeletal and smooth muscle (Ebashi, 1976; Endo, 1977; Kuriyama *et al.*, 1982), increased the amount of stored Ca^{2+} estimated using caffeine to about double that of the control value (Itoh *et al.*, 1981). However, in the cat trachea, procaine (10 mM) increased only slightly the stored Ca (up to 1.3 times of the control value), thereby indicating that the storage sites in the cell may be almost saturated with Ca^{2+}, under physiological conditions. This would explain why contractions evoked by e.j.p. or phasic response evoked by exogenous ACh are larger and resistant to Ca^{2+}-free EGTA-containing solution than are those evoked by electrical membrane depolarization or excess concentrations of $[K]_0$.

In the cat trachea, neither depolarizing current pulses applied to the smooth muscle cells nor field stimulation to the excitatory neurones evoked an action potential, as noted for bovine or dog tracheal smooth muscles (Suzuki *et al.*, 1976; Kirkpatrick, 1981; Ito & Tajima, 1981). In the presence of TEA or procaine, however, depolarizing current-pulses lead to generation of an action potential, as is the case in the dog trachea (Suzuki *et al.*, 1976, Ito & Tajima, 1982). TEA greatly enhances the contractile response, while procaine abolishes the mechanical response. Therefore, it seems likely, that in the presence of procaine, Ca ions entering during the action potential do not directly activate the contractile proteins, but that Ca^{2+} entering during the spike electrogenesis is sequestered in storage sites in the muscle cells of the trachea. The initiation of contraction in the cat trachea might thus be due to Ca^{2+}-induced Ca^{2+} release mechanisms, as proposed for the guinea-pig mesenteric artery (Itoh *et al.*, 1981).

These physiological characteristics of smooth muscle cells of the cat trachea would explain why the potent vasodilators (including nifedipine, verapamil and diltiazem) have a limited effect in alleviating bronchial constriction (Himori & Taira, 1980; Nagao *et al.*, 1981).

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