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Time course of Ca^{2+}-dependent K^+ and Cl^- currents in single smooth muscle cells of guinea-pig trachea

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Abstract

The time course of two types of Ca^{2+}-dependent currents were compared in single smooth muscle cells freshly isolated from guinea-pig trachea. When the pipette solution contained mainly 140 mM KCl, depolarization from −60 mV to 0 mV evoked an initial inward current followed by an outward current which consisted of transient (I_{to}) and sustained components. In addition, a long-lasting inward tail current (I_{tail}) was occasionally observed after the repolarization to −60 mV. Although I_{to} often occurred repetitively during depolarization, the first I_{to} reached the peak of ~50 ms after the start of depolarization and had the largest amplitude in most cells examined. The amplitude of I_{tail} increased with the increase in depolarization period up to about 500 ms. Pharmacological analyses indicate that I_{to} and I_{tail} are Ca^{2+}-dependent K^+ and Cl^- currents ($I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$), respectively, and suggest that not only Ca^{2+}-influx through Ca^{2+} channels but also subsequent Ca^{2+} release from stores contributes to activate these currents. Spontaneous transient outward and inward currents, $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$, respectively, were simultaneously recorded at −40 mV. In over 80% of the spontaneous current events, outward and inward currents coupled one to one and always occurred in this order. Puff-application of 10 mM caffeine also induced $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ in this order at −40 mV. When caffeine was applied twice with various intervals, the current amplitude in the second application depended upon the period of the interval. The recovery of $I_{Cl\text{-}Ca}$ during the interval was faster than that of $I_{K\text{-}Ca}$. The results indicate that the activation and decay time courses of $I_{Cl\text{-}Ca}$ are slower but its recovery is faster than those of $I_{K\text{-}Ca}$.

Keywords: K^+ channel, Ca^{2+}-dependent; Cl^- channel, Ca^{2+}-dependent; Patch clamp; Smooth muscle cell; Trachea

1. Introduction

The activation of some types of ion channels in smooth muscle cells is associated with an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The trigger for the elevation of $[Ca^{2+}]_i$ can be either Ca^{2+} release from an intracellular Ca^{2+} store, presumably located in the sarcoplasmic reticulum, or Ca^{2+} influx from the extracellular space through voltage-dependent Ca^{2+} channels (Ohya et al., 1987; Pacaud et al., 1989a).

Characteristics of Ca^{2+}-dependent K^+ current ($I_{K\text{-}Ca}$) and its modulation by bioactive substances have been extensively studied in smooth muscle cells, since the current is one of the major membrane currents in both electrophysiologically active and quiescent smooth muscles (see as a review; Bolton and Beech, 1992). In quiescent smooth muscle tissues including trachea, strong outward rectification of membrane conductance upon depolarization has been considered to be attributable to its low membrane excitability (Stephens and Kroeger, 1970). In single tracheal smooth muscle cells, it has been reported that membrane depolarization activates large $I_{K\text{-}Ca}$ and delayed rectifier K^+ current ($I_{K\text{-}D}$) in contrast to the relatively small voltage-dependent Ca^{2+} current (Hisada et al., 1990; Muraki et al., 1990). Suppression of K^+ currents by blockers reduces the outward rectification and increases membrane excitability (Kirkpatrick, 1975; Imaizumi and Watanabe, 1981).

Ca^{2+}-dependent Cl^- current ($I_{Cl\text{-}Ca}$) has been recorded in various smooth muscles; rabbit portal vein (Byrne and Large, 1988), ear artery (Amédéé et al., 1990), esophagus (Akbarali and Giles, 1993), rat ileum (Ohta et al., 1993), portal vein (Pacaud et al., 1989a, Pacaud et al., 1989b), guinea-pig trachea (Janssen and Sims, 1992), porcine and rabbit coronary artery (Klöckner and Isenberg, 1991; Lamb et al., 1994). The amplitude of the current, however,

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appears to be varied widely in smooth muscle cells from different tissues. Elevation of $[Ca^{2+}]_i$ resulting from Ca^{2+} release from intracellular Ca^{2+} stores activates both $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$. These currents are outward and inward currents, respectively, at resting membrane potentials of about −60 mV, which may be approximately half-way between their reversal potentials of about −80 and −30 mV, respectively. The physiological functions of $I_{Cl\text{-}Ca}$ remains unclear in comparison with those of non-selective cationic current which directly elicits depolarization with a larger electromotive force and triggers action potentials in some smooth muscle cells (for a review, see Inoue and Chen, 1993).

In canine and bovine tracheal smooth muscle cells, contraction induced by acetylcholine, histamine or serotonin is mainly mediated by the formation of inositol-trisphosphate and the subsequent Ca^{2+} release from sarcoplasmic reticulum, namely pharmaco-mechanical coupling (Coburn and Yamaguchi, 1977; Farley and Miles, 1977). Small contribution of membrane depolarization induced by high concentration of acetylcholine to contraction has been suggested. Larger contribution of membrane potential changes to tension development has, however, been suggested in guinea-pig trachea, where large oscillation of membrane potential occurs in the presence of agonists (Small, 1982). Moreover, it has been shown that the contribution of $I_{Cl\text{-}Ca}$ to inward current induced by histamine or substance P is much larger than non-selective cationic current in guinea-pig tracheal smooth muscle cells (Janssen and Sims, 1993, 1994). $I_{Cl\text{-}Ca}$ may, therefore, possibly be important especially in guinea-pig tracheal smooth muscle cells, whereas little is known about the characteristics of $I_{Cl\text{-}Ca}$ activation, including the time course.

The present study was undertaken to elucidate the difference between activation time course of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ in tracheal smooth muscle cells of the guinea-pig. Spontaneous transient outward and inward currents, $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$, respectively, were simultaneously recorded and their time courses of activation were also compared.

2. Materials and methods

2.1. Cell isolation

Male Hartley guinea-pigs, weighing about 200 g, were killed by a blow to the head and the trachea was dissected. Single cells were dispersed using collagenase and papain as described previously (Muraki et al., 1990; Henmi et al., 1995).

2.2. Electrophysiological measurements in single cells

Membrane currents were recorded using the standard whole-cell voltage clamp methods (Hamill et al., 1981) and an EPC7 (List, Germany) or CEZ-2300 (Nihon-koden, Japan) amplifier. In some experiments, the nystatin-perforated method was used (Horn and Marty, 1988). Current and voltage signals were stored and analyzed as described previously (Imaizumi et al., 1990). All experiments were carried out at 23 ± 2°C. When nystatin-perforated method was used, the membrane potential was corrected for the junction potential by −2 mV.

Micro-application of caffeine was performed by puff with pressure-ejection from a pipette filled with extracellular medium plus caffeine in the same way described previously (Henmi et al., 1995).

2.3. Solution

The standard bathing solution contained (mM): NaCl 137, KCl 5.9, $CaCl_2$ 2.2, $MgCl_2$ 1.2, glucose 14, HEPES 10. The pH was adjusted to 7.2 by NaOH. The pipette-filling solution had following composition (mM); KCl 140, $MgCl_2$ 4, Na_2ATP 5, HEPES 10, EGTA 0.05. The pH was adjusted to 7.2 by KOH. When nystatin-perforated patch-clamp method was used, the pipette-filling solution contained (mM); KCl 140, $MgCl_2$ 4, HEPES 10, nystatin (200 ~ 600 μg/ml). Nystatin was dissolved in dimethylsulfoxide (DMSO) before use. The pH was adjusted to 7.2 by KOH. When the outward current was inhibited, K^+ was replaced with equimolar Cs^+.

2.4. Drugs

Caffeine, $CdCl_2$, dimethylsulfoxide (DMSO), CsCl, choline chloride and dithiothreitol were obtained from Wako Pure Chemical (Osaka, Japan). Niflumic acid, cyclopiazonic acid, nystatin, adenosine triphosphate disodium salt (Na_2ATP), papain were obtained from Sigma (St. Louis, MO, USA). Bovine serum albumin (MILES, Fraction V), collagenase (500 unit/mg Yakult, Tokyo, Japan), tetraethylammonium chloride (TEA) (Tokyo Chemical Industry, Tokyo, Japan), iberiotoxin (Peptide Institute, Osaka, Japan), EGTA (Dojin Kagaku, Kumamoto, Japan) and atropine sulfate (Nakalai Tesque, Tokyo, Japan) were from the companies shown in parentheses.

2.5. Statistics

Results were expressed as means ± S.E.M. Statistical significance was examined using a paired or unpaired Student's t-test. $P < 0.05$ was considered significant, indicated in the figures by an asterisk; P values are presented after adjustment for multiple comparisons with the Bonferroni correction.

3. Results

3.1. Membrane currents upon depolarization in tracheal smooth muscle cells

Membrane currents were recorded in single smooth muscle cells isolated from trachea in the standard bathing solution. When the pipette solution contained mainly 140 mM KCl, a step depolarization from the holding potential of −60 mV to 0 mV elicited a small transient inward current and a subsequent outward current which consisted of transient (I_{to}) and sustained components. The most typical example is shown in Fig. 1Aa. The initial inward current was a voltage-dependent Ca^{2+} channel current (I_{Ca}), since the addition of 0.3 mM cadmium (Cd^{2+}) to the extracellular solution abolished the current (not shown, see Fig. 2). The amplitude and shape of I_{to} varied widely from cell to cell. Occasionally, I_{to} occurred repetitively during depolarization for 150 ms (Fig. 1Ab), whereas the first I_{to} just after the start of depolarization had the largest amplitude in most cells examined (see also Fig. 3). In some cells, the activation of delayed rectifier outward current was clearly observed and a large I_{to} occurred several hundred ms after the start of depolarization (Fig. 1Ac). Characteristics of the delayed rectifier outward current was not further examined in this study. These I_{Ca} and outward currents upon depolarization were similar to those observed in tracheal smooth muscle cells of the dog (Muraki et al., 1990) and guinea-pig (Hisada et al., 1990). In addition, long-lasting inward tail current (I_{tail}), which has not been reported in previous papers, was often observed after returning to the holding potential (Fig. 1). The amplitude of I_{tail} just after the repolarization varied even more widely from cell to cell than that of peak I_{to}. Although there appeared to be a positive correlation between the amplitude of I_{to} and I_{tail}, only a small I_{tail} was observed in some cells where a large I_{to} was recorded (see Fig. 3A,a). The mean amplitude of I_{Ca}, I_{to} and I_{tail} were -28.8 ± 5.5 pA, $+470.6 \pm 120.1$ pA and -119.9 ± 20.3 pA ($n = 10$), respectively, when cells were depolarized from −60 to 0 mV for 150 ms once every 15 s.

Fig. 1B shows results of experiments in which the duration of the depolarizing pulse was increased from 30 to 500 ms to observe the activation time course of I_{tail}. The pipette solutions contained mainly KCl (a) and CsCl (b), respectively. The outward current shown in Fig. 1Ba is the average of eleven traces during depolarization. While I_{to} reached the peak after about 50 ms from the start of the depolarization, the amplitude of I_{tail} was increased with the increase in duration up to 300 ms and was maintained or slightly decreased by a further increase in duration (Fig. 1Ba). The slow activation time course of I_{tail} was even clearer when K^+ currents were blocked by internal Cs^+ (Fig. 1Bb). I_{Ca} was also clearly observed under these conditions. It is notable that, when the depolarization period was 150 and 200 ms, I_{tail} increased after repolarization and reached the peak after about 30–50 ms (see also Fig. 2Ab). This may suggest that I_{tail} does not simply show the deactivation time course of voltage-dependent current which was activated during the depolarization.

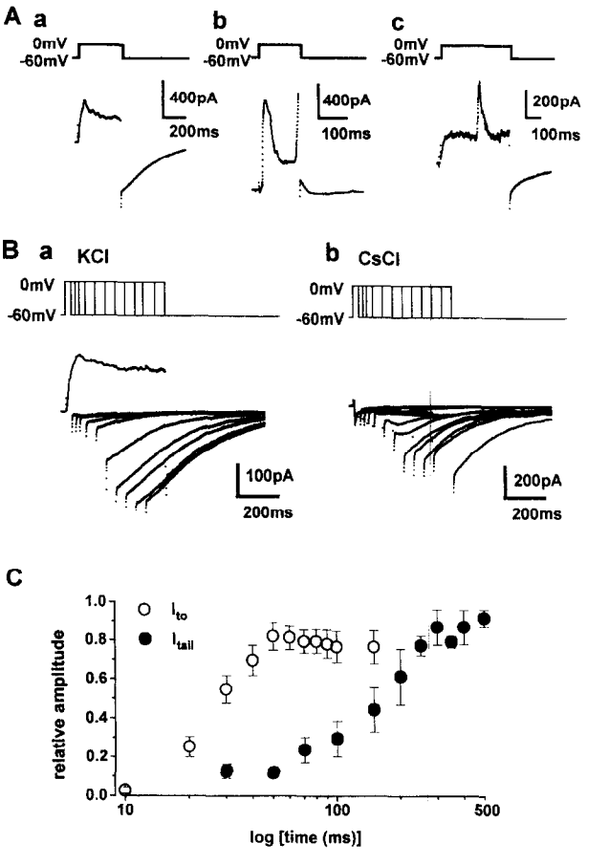

Fig. 1. Activation time courses of currents upon depolarization in tracheal smooth muscle cells. (A) Different types of current shapes in three separate cells activated by depolarization from −60 to 0 mV once every 15 s. The pipette solution contained mainly KCl. A transient outward current (I_{to}) was activated just after the depolarization in most cells examined as shown in 'a' and 'b'. Occasionally I_{to} occurred after several hundred ms delay from the start of depolarization (c) or occurred repetitively during depolarization (b). The shape of outward current shown in 'a' is the most typical under the experimental conditions (6 out of 12 cells). Slow inward tail current after repolarization to −60 mV (I_{tail}) was shown in 'a' and 'c'. Such large I_{tail}s were observed in 12 out of 31 cells. (B) The duration of depolarization was changed to elucidate the activation time courses of I_{to} and I_{tail}. The cells were depolarized from −60 to 0 mV every 15 s. The duration was changed from 30 to 500 ms. Traces in 'a' and 'b' are recorded using KCl and CsCl rich pipette solutions, respectively. (C) Summarized data obtained from experiments typically shown in 'B'. Averaged values of the relative amplitude of I_{to} and I_{tail} during their activation were plotted against the time after the start of depolarization (I_{to}) or the duration of depolarization (I_{tail}). The activation time course of I_{to} up to 50 ms was well fitted by a single exponential function (time constant: 25.5 ± 5.3 ms). The time course of the increase in I_{tail} was not described by single exponential or sum of two exponential functions. Note that the activation of I_{tail} was markedly slower than that of I_{to}.

Fig. 1C illustrates the summarized results of activation of I_{to} and I_{tail} in the same conditions shown in Fig.

1Ba,Bb. The relative amplitude of I_{to} and I_{tail} was plotted against depolarization period in logarithmic scale. The maximum amplitude of I_{to} or I_{tail} was taken as unity in measuring the relative current amplitude in each cell. The amplitude of I_{to} increased exponentially with a time constant of 25.5 ± 5.3 ms ($n = 7$) and reached the maximum about 50 ms after the start of depolarization. Since the decay time course of I_{to} varied widely from pulse to pulse in a cell and another I_{to} occasionally occurred during 150 ms depolarization, 11 or 12 traces of I_{to} in the protocol were averaged in each cell. When a pipette solution containing mainly KCl was used, I_{tail} was often seriously contaminated by tail current of I_{to} (Fig. 1Ab). Although the activation time course of I_{tail} was not simple, it is clear that the activation reached maximum 200–300 ms after the start of depolarization in most cells and was much slower than that of I_{to}. It is notable that the amplitude of I_{tail} increased with the increase in depolarization duration even after I_{to} reached the peak and started to decline. These results indicate that the activation mechanism of I_{tail} is distinctive from that of I_{to}.

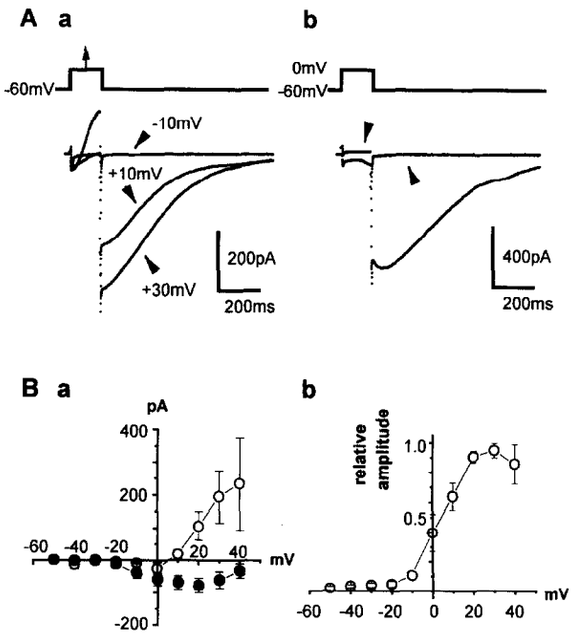

Fig. 2. Characteristics of slowly activating Cl^- current during depolarization and its tail current. (A) In 'a', the cell was depolarized from −60 mV to −10, +10 and +30 mV for 150 ms once every 15 s. When the cell was depolarized to +10 or +30 mV, the current was inward first and reversed outward during depolarization. Large inward tail currents were observed upon repolarization. The potentials indicated by arrows show the preceded depolarization corresponding to the tail currents. In 'b', the cell was depolarized from −60 to 0 mV. Both the current during depolarization and following I_{tail} were blocked by the addition of 0.3 mM Cd^{2+} as indicated by arrows. The pipette solution contained mainly CsCl in both 'a' and 'b'. (B) The current-voltage relationships obtained from experiments typically shown in 'A,a' are illustrated in 'a'. ●: The peak amplitude of the initial inward current (I_{Ca}). ○: The amplitude at the end of depolarization, $n = 5$. The reversal potential of the current at the end of depolarization was 4.5 ± 3.9 mV. In 'b', the relative amplitude of I_{tail} upon repolarization was plotted against the preceded depolarizing potentials. The maximum I_{tail} was taken as unity (1.0). The data was fitted by the Boltzmann equation. The half activation potential and the slope factor were 5.21 ± 2.73 mV and 7.81 ± 2.46 mV ($n = 5$), respectively.

Although the I_{to} upon depolarization has been reported as Ca^{2+}-dependent K^+ current in tracheal smooth muscle cells (Hisada et al., 1990; Muraki et al., 1990), the current responsible for the I_{tail} has not been identified yet. Fig. 2A shows the current at various depolarizing potentials and the subsequent I_{tail}s after repolarization to −60 mV under the conditions where K^+ currents were blocked by internal Cs^+. The depolarizing potential was increase step-wise by 10 mV from −60 mV. At −10 mV or lower potentials, the current during depolarization was continuously inward. The inward current at −10 or 0 mV was somewhat complicated; I_{Ca} was activated first and occasionally increased again after about 100 ms (Fig. 2Ab). At potentials positive to +10 mV, the current during depolarization reversed from inward to outward. Large I_{tail}s were recorded after repolarization to −60 mV when the depolarizing potential was positive to 0 mV. Application of 0.3 mM Cd^{2+} abolished all currents; I_{Ca}, the late inward or outward current during depolarization and the I_{tail} (Fig. 2Ab). Fig. 2Ba illustrates the current-voltage relationships between the depolarizing potentials and the peak amplitude of initial I_{Ca} current (closed circles) or the current amplitude at the end of 150 ms pulse (open circles). Averaged reversal potential of the current at the end of pulse was 4.5 ± 3.9 mV ($n = 5$). In Fig. 2Bb, the relative amplitude of I_{tail} normalized by the maximum was plotted against the potential of depolarization. The dependence of I_{tail} on the activation potentials was well described by the Boltzmann equation. The half activation potential and the slope factor were 5.2 ± 2.7 mV and 7.8 ± 2.5 mV ($n = 5$), respectively.

3.2. Pharmacological characteristics of currents elicited by depolarization

To investigate pharmacological characteristics of I_{to} and I_{tail} in tracheal smooth muscle cells, effects of various agents were examined. The pipette solution contained mainly 140 mM KCl (Fig. 3A) or CsCl (Fig. 3B). Application of 100 nM iberiotoxin markedly reduced the outward current upon depolarization from −60 to 0 mV (Fig. 3Aa). Further application of 2 mM TEA slightly reduced the sustained component of outward current, whereas effects of a single application of 2 mM TEA were almost identical to those of 100 nM iberiotoxin and 2 mM TEA (not shown). The transient component (I_{to}) was particularly susceptible to these blockers, suggesting that this current is due to the activation of large conductance Ca^{2+}-dependent K^+ channels. Addition of 10 μM niflumic acid, Cl^- channel blocker, inhibited I_{tail} (Fig. 3Ba), suggesting that I_{tail} is due to the activation of Cl^- channels. Moreover, the addition of 0.3 or 1.0 mM Cd^{2+} abolished I_{to} as well as

I_{tail} and I_{Ca} (not shown, $n = 3$), indicating that activation of I_{to} and I_{tail} requires Ca^{2+} influx or elevation of $[Ca^{2+}]_i$. When the pipette solution contained 10 mM EGTA, neither I_{to} nor I_{tail} was observed (not shown, $n = 3$). The amplitude of I_{tail} was not affected by replacing 126 mM Na^+ in the external solution with equimolar TEA ($n = 3$) or choline plus 3 μM atropine ($n = 2$). These results indicate that I_{to} and I_{tail} upon depolarization are Ca^{2+}-dependent K^+ and Cl^- currents, respectively.

Fig. 3Ab,Bb show that application of 10 mM caffeine inhibited I_{to} and I_{tail}. Application of 10 μM cyclopiazonic acid, a selective inhibitor of sarco-/endo-plasmic reticulum Ca^{2+}-ATPase, also markedly reduced I_{tail} as well as I_{to} (Suzuki et al., 1992) (Fig. 3Ac,Bc). These effects of caffeine and cyclopiazonic acid were removed by washout (not shown, $n = 11$ and 4 for each). Caffeine and cyclopiazonic acid do not directly affect large conductance Ca^{2+}-dependent K^+ channel in excised patch from smooth muscle cells (caffeine: Muraki et al., 1992; cyclopiazonic acid: Suzuki et al., 1992), whereas their direct action on Ca^{2+}-dependent Cl^- channels has not been examined yet. It can be, therefore, assumed that activation of I_{to} and I_{tail} upon depolarization are due not only to entered Ca^{2+} through voltage-dependent Ca^{2+} channels but also released Ca^{2+} from intracellular storage sites via Ca^{2+}-induced Ca^{2+} release mechanism triggered by the Ca^{2+} entry.

3.3. Spontaneous transient currents in tracheal smooth muscle cells

As reported in the previous paper (Henmi et al., 1995), spontaneous transient inward currents were observed in about ~15% of cells examined, whereas spontaneous transient outward currents were observed in over 80% cells. Based upon pharmacological characteristics, it is very likely that these outward and inward currents are due to the activation of Ca^{2+}-dependent K^+ and Cl^- channels, respectively, resulting from periodical Ca^{2+} release from intracellular Ca^{2+} storage sites in tracheal smooth muscle cells (Janssen and Sims, 1994; Henmi et al., 1995). In contrast to the previous observation (Janssen and Sims, 1994), spontaneous outward currents were markedly reduced by 30 nM iberiotoxin in the present study (Fig. 4Aa; $n = 6$), indicating that activation of large conductance Ca^{2+}-dependent K^+ channels is responsible for the current. The block by iberiotoxin was partly removed by washout. Spontaneous inward currents were blocked by 10 μM niflumic acid (Fig. 4Ab; $n = 3$).

When the pipette solution contained mainly KCl, the reversal potentials of spontaneous transient outward and inward currents were assumed to be approximately −80 and 0 mV, respectively. At a holding potential of −40 mV, the electromotive force of the outward and inward currents may be roughly equal. In a cell where spontaneous inward currents were observed, the outward and inward currents which coupled in a one to one manner were frequently recorded at −40 mV as shown in Fig. 4B. Fig. 4C illustrates summarized data that the occurrence of coupled outward and inward currents was 85.3 ± 3.5% out of 179 events from 3 cells. Occasionally, only the outward (10.0 ± 6.2%) or inward current (4.8 ± 2.6%) was observed as a single event. In coupled currents, the deflection was always first outward and then inward (100% out of 153 events). At a holding potential of −60 mV, similar

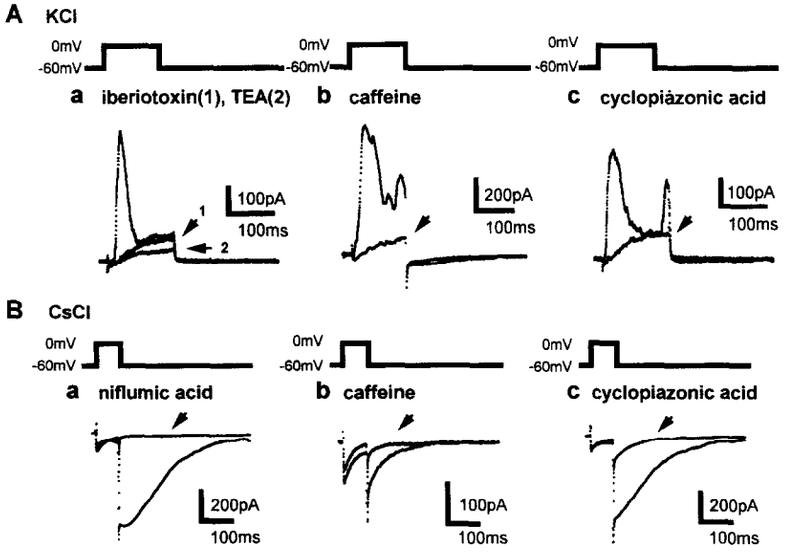

Fig. 3. Pharmacological characteristics of currents elicited by depolarization. The cell was depolarized from the holding potential of −60 mV to 0 mV. The pipette solution contained mainly 140 mM KCl in 'A' and 140 mM CsCl in 'B'. Current traces recorded in the presence of drugs were indicated by arrows. (A) Application of 100 nM iberiotoxin (indicated by '1'), 2 mM TEA (indicated by '2'), 10 mM caffeine and 10 μM cyclopiazonic acid almost inhibited the transient outward current (I_{to}) upon depolarization. (B) Replacement of K^+ with Cs^+ in the pipette solution inhibited outward currents but not the long-lasting inward tail current (I_{tail}). Application of 10 μM niflumic acid, 10 mM caffeine and 10 μM cyclopiazonic acid inhibited I_{tail} but not the transient inward current, which was voltage-dependent Ca^{2+} current.

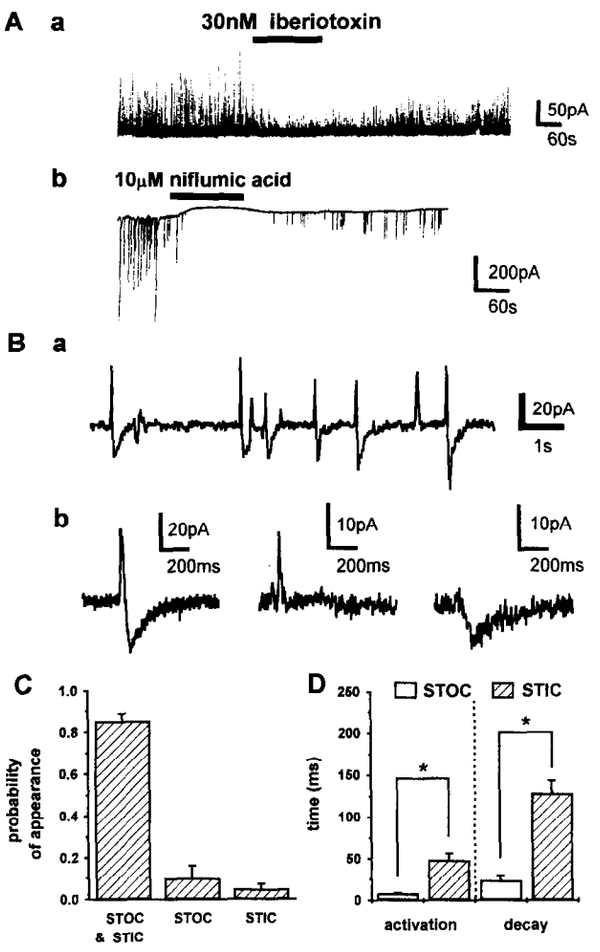

Fig. 4. Time courses of spontaneous transient currents. (A) Spontaneous transient outward currents recorded at the holding potentials of 0 mV using KCl rich solution were markedly reduced by the application of 30 nM iberiotoxin and partly recovered by washout (a). Spontaneous transient inward currents recorded at the holding potential of −60 mV using CsCl rich solution were completely blocked by 10 μM niflumic acid and partly recovered by washout (b). (B) Spontaneous transient outward and inward currents were often observed as a coupled event at the holding potential of −40 mV, when the pipette solution contained mainly 140 mM KCl. Note that an outward deflection was followed by an inward one in most events (a). A coupled outward and inward currents (left in 'b') and independent outward (middle) and inward (right) currents were recorded in faster time-scale. (C) The probability of appearance of coupled spontaneous transient outward and inward currents (STOC and STIC) and independent outward (STOC) or inward currents (STIC) such as shown in 'B,b, left, middle and right panels', respectively, were determined from 179 spontaneous events in three cells. The spontaneous events were recorded at −40 mV. Note that, in most events, an outward current was followed by an inward current. (D) The activation time of independent outward (STOC, open column, $n = 6$) and inward current (STIC, hatched column, $n = 5$) was measured as the time to peak from the 10% level of the maximum amplitude from the base ('activation' in abscissa). The decay time was shown as the time from the peak to the 10% level from the base ('decay' in abscissa). The activation time and the decay time of the outward currents are significantly smaller than those of the inward currents, respectively ($P < 0.05$).

results were obtained ($n = 3$), whereas the outward and inward currents were smaller and larger than at −40 mV, respectively. Fig. 4D shows the time to peak from the 10% level of the maximum amplitude from the base (activation) and also the time from the peak to the 10% level from the base (decay) of the outward and inward currents which occurred independently as single events. In the independent outward and inward currents, the activation time was 8.2 ± 0.7 ($n = 6$) and 48.4 ± 7.6 ms ($n = 5$, $P < 0.05$),

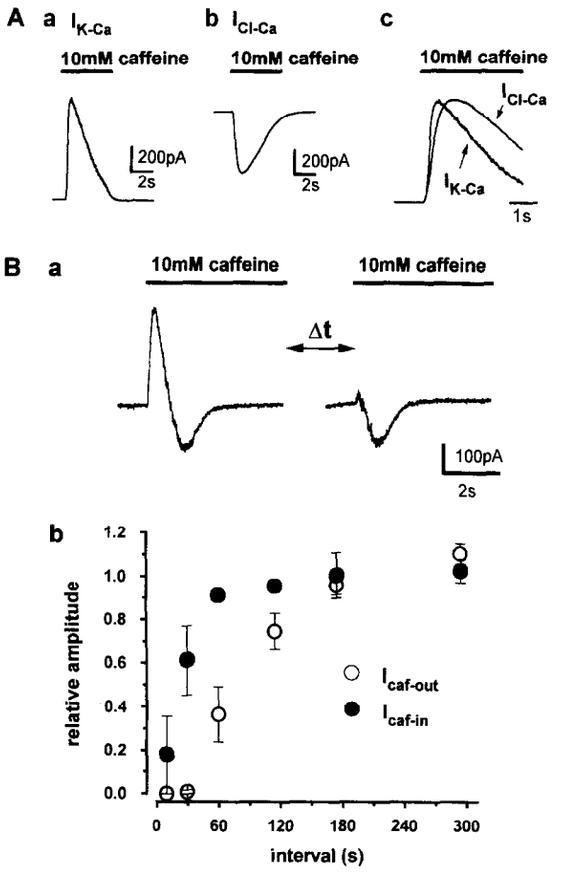

Fig. 5. Time course of caffeine-induced current. (A) $I_{K\text{-}Ca}$ or $I_{Cl\text{-}Ca}$ was recorded in a cell by the application of 10 mM caffeine at the holding potential of −40 mV in the presence of 10 μM niflumic acid (a) and 126 mM TEA (b), respectively. The pipette solution contained mainly 140 mM KCl. Caffeine was puff-applied for 5 s by pressure ejection from a pipette which was filled with external solution containing 10 mM caffeine. The two recordings in 'a' and 'b' were superimposed in 'c' after adjusting the maximum amplitude on the computer. Note that both the activation and the decay of $I_{K\text{-}Ca}$ are faster than those of $I_{Cl\text{-}Ca}$, respectively. (B) A paired puff-application of caffeine was performed once every 5 min at the holding potential of −40 mV (a). The first application of caffeine induced outward current which reversed to inward during the application (a). The interval between applications in a pair was changed from 10 to 295 s. The relative amplitude of outward or inward peak in the latter application of a pair was normalized by that of the former one, respectively, and plotted against the interval (b). Open and closed circles indicate the mean of relative amplitude of the peak outward and inward currents ($I_{caf\text{-}out}$ and $I_{caf\text{-}in}$), respectively. The half-recovery time of $I_{caf\text{-}in}$ and $I_{caf\text{-}out}$ was 15.8 ± 4.9 and 71.1 ± 10.4 s ($n = 4$, $P < 0.01$), respectively.

respectively. The decay time was 23.5 ± 6.0 ($n = 6$) and 128.4 ± 14.6 ms ($n = 5$, $P < 0.05$), respectively. It is clear that activation and decay time courses of the inward currents are significantly slower than those of outward currents. It is notable that, if independent outward and inward currents in Fig. 4Bb are added, the shape of resulting current is quite close to that of coupled current.

3.4. Time course of caffeine-induced current

$I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ were also elicited by Ca^{2+} release induced by 10 mM caffeine in tracheal smooth muscle cells of the guinea-pig (Henmi et al., 1995). Fig. 5Aa,b show time courses of $I_{K\text{-}Ca}$ or $I_{Cl\text{-}Ca}$ induced by puff-application of 10 mM caffeine in a cell where $I_{Cl\text{-}Ca}$ or $I_{K\text{-}Ca}$ was blocked by 10 μM niflumic acid or 126 mM TEA, respectively. In Fig. 5Ac, $I_{Cl\text{-}Ca}$ in 'A' were reversed and superimposed on $I_{K\text{-}Ca}$ and their peak amplitude was adjusted on the computer. It is clear that the time to peak of $I_{Cl\text{-}Ca}$ is about 0.8 s slower than that of $I_{K\text{-}Ca}$ and that the difference in decay time courses of them was even larger.

In the previous paper, the relationship between caffeine-induced $I_{Cl\text{-}Ca}$ and Ca store sites was examined using CsCl rich pipette solution (Henmi et al., 1995). It was shown that, when 10 mM caffeine was applied twice at various intervals, the amplitude of the latter $I_{Cl\text{-}Ca}$ strongly depended upon the interval. The longer the interval, the larger the amplitude of the latter $I_{Cl\text{-}Ca}$, indicating that the recovery of $I_{Cl\text{-}Ca}$ was due to refilling of stores with Ca^{2+} during the interval. In the present study, $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ were recorded with KCl-rich pipette solution using the same protocol (Fig. 5Ba). A pair of puff application of 10 mM caffeine for 5 s was performed once every 5 min. The averaged peak amplitude of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ by the first puffing of 10 mM caffeine was 478.6 ± 55.2 and −483.3 ± 38.7 pA ($n = 8$, $P < 0.05$) and the time to peak from the start of puff was 1.35 ± 0.85 and 5.34 ± 0.96 s ($n = 8$; $P < 0.05$), respectively. Surprisingly, $I_{K\text{-}Ca}$ was markedly smaller than $I_{Cl\text{-}Ca}$ in the latter puffing, when the interval was short (30 s; Fig. 5Ba). Summarized data of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ recovery time courses are shown in Fig. 5Bb. It is notable that the recovery of $I_{Cl\text{-}Ca}$ was significantly faster than that of $I_{K\text{-}Ca}$; the half recovery time was 15.8 ± 4.9 and 71.1 ± 10.4 s ($n = 4$; $P < 0.01$), respectively.

4. Discussion

The present study clearly shows that membrane depolarization elicits two types of Ca^{2+}-dependent currents; $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$, in addition to other voltage-dependent currents in guinea-pig tracheal smooth muscle cells. Since the outward current elicited by depolarization to 0 mV was markedly reduced by external addition of 0.3 mM Cd^{2+}, 2 mM TEA or 100 nM iberiotoxin or internal application of 10 mM EGTA, the major component is through large conductance Ca^{2+}-dependent K^+ channels, as has been reported in smooth muscle cells from various tissues including trachea (Muraki et al., 1990, 1992).

Based upon the reversal potential of the slowly activating current during depolarization, the current and its I_{tail} were mainly $I_{Cl\text{-}Ca}$ when K^+ currents were blocked by internal Cs^+. Although it has been well established that Ca^{2+} release from intracellular storage sites by agonists such as acetylcholine, histamine and caffeine induces $I_{Cl\text{-}Ca}$ in tracheal smooth muscle cells, $I_{Cl\text{-}Ca}$ upon depolarization and its characteristics were first shown here. The pharmacological analyses using Cd^{2+}, caffeine and cyclopiazonic acid strongly suggest that $I_{Cl\text{-}Ca}$ upon depolarization in tracheal cells is activated not only by Ca^{2+} influx through voltage-dependent Ca^{2+} channels but also by the subsequent Ca^{2+}-induced Ca^{2+} release from storage sites as has been suggested in smooth muscle cells of the coronary artery (Lamb et al., 1994).

In the present study, $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ were also observed as spontaneous transient outward and inward currents, respectively. The generation of these currents may be due to a spontaneous and random Ca^{2+} release from sarcoplasmic reticulum in local areas of a cell (Benham and Bolton, 1986; Janssen and Sims, 1994; Henmi et al., 1995). It was suggested that spontaneous transient outward currents in guinea-pig tracheal smooth muscle cells were not due to activation of large conductance Ca^{2+}-dependent K^+ channels (Janssen and Sims, 1994). It is, however, confirmed that 30 nM iberiotoxin markedly reduced the currents, suggesting strongly that the activation of large conductance Ca^{2+}-dependent K^+ channels is responsible for the spontaneous outward currents.

Of importance is that spontaneous outward and inward currents did not occur independently but were observed as a coupled current in over 85% of the spontaneous transient events and that the outward deflection did always precede the inward one in each coupled event. The results suggest that a local Ca^{2+} release may stimulate simultaneously Ca^{2+}-dependent K^+ and Cl^- channels in a small area of cell membrane nearby but the activation of Cl^- channel is much slower than that of K^+ channel. The deactivation of the Cl^- channel may be also slower than that of K^+ channel. The slower time course of $I_{Cl\text{-}Ca}$ than that of $I_{K\text{-}Ca}$ was also clearly observed when these currents were elicited by depolarization. The activation of $I_{K\text{-}Ca}$ reached the maximum at about 50 ms from the start of depolarization to 0 mV, whereas the activation of $I_{Cl\text{-}Ca}$ started approximately at the peak of $I_{K\text{-}Ca}$. The slower activation of $I_{Cl\text{-}Ca}$ than that of $I_{K\text{-}Ca}$ was also observed when these currents were elicited by puff-application of caffeine. Several possibilities can be considered as the cause of such a large difference between time course of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$.

Firstly, the sensitivity of Ca^{2+}-dependent Cl^- channel to Ca^{2+} may be lower than that of large conductance Ca^{2+}-dependent K^+ channel. Such possibility has been

reported in lacrimal gland cells (Marty et al., 1984; Mayer, 1985). It has been reported that the gating of Ca^{2+}-dependent Cl^- channels is mainly controlled by $[Ca^{2+}]_i$ in portal vein myocytes and that the threshold of $[Ca^{2+}]_i$ for activation of $I_{Cl\text{-}Ca}$ is 180 nM (Pacaud et al., 1992). The faster recovery of $I_{Cl\text{-}Ca}$ than that of $I_{K\text{-}Ca}$ during the interval of paired application of caffeine is a striking observation and apparently do not fit the idea that Ca^{2+} sensitivity of the Cl^- channel is lower than that of the K^+ channel. Since intrinsic inactivation of the K^+ channel has not been observed in smooth muscle cells, this result rather suggests the opposite possibility. Exact Ca^{2+}-sensitivity of single Ca^{2+}-dependent Cl^- channel, however, remains unclear yet, since the density of the Cl^- channel in smooth muscle cells is much sparser than that of the K^+ channels and the activity runs down in excised patch (Klöckner, 1993; Renterghem and Lazdunski, 1993; White et al., 1995). Even if the Ca^{2+} sensitivity of the Cl^- channel is lower than that of the K^+ channel, the slower time course of $I_{Cl\text{-}Ca}$ than that of $I_{K\text{-}Ca}$ may not be easily explained (Hogg et al., 1993).

Secondly, the source of Ca^{2+} for activation of $I_{K\text{-}Ca}$ may be different from that of $I_{Cl\text{-}Ca}$. $I_{K\text{-}Ca}$ may be activated directly by Ca^{2+} influx and $I_{Cl\text{-}Ca}$ may be mediated by Ca^{2+} release from storage sites via Ca^{2+}-induced Ca^{2+} release mechanism. Alternatively, $I_{K\text{-}Ca}$ might be activated by Ca^{2+} release from stores distinctive from those for $I_{Cl\text{-}Ca}$ activation. The generation of spontaneous transient outward/inward currents and caffeine-induced currents, however, does not strongly depend upon Ca^{2+}-influx (Henmi et al., 1995). It is also shown clearly that, in over 85% of spontaneous current events, outward and inward currents coupled in this order, suggesting local Ca^{2+} release stimulates simultaneously both channels in a limited area of cell membrane but activation of K^+ channels is always faster than that of Cl^- channels. Moreover, Ca^{2+}-induced Ca^{2+} release mechanism may be involved in the generation of I_{to} upon depolarization as well as that of I_{tail}, since caffeine and cyclopiazonic acid markedly reduced these currents in a similar manner. Therefore, the possibility appears to be unlikely.

Thirdly, activities of Ca^{2+}-dependent K^+ and Cl^- channels may be simply regulated by $[Ca^{2+}]_i$ but the kinetics of the Cl^- channel is much slower than that of the K^+ channel. The slower kinetics of spontaneous inward current than those of the outward current have been extensively analyzed in vascular smooth muscle cells (Hogg et al., 1993; Wang et al., 1992). The large conductance Ca^{2+}-dependent K^+ channel gating in smooth muscle cells is usually quite fast (Markwardt and Isenberg, 1992). Therefore, the time course of spontaneous outward current may directly reflect the time course of $[Ca^{2+}]_i$ at the inner surface of the cell membrane (Hogg et al., 1993). The activation of $I_{Cl\text{-}Ca}$ during depolarization is not particularly slow in comparison with the two types of delayed rectifier K^+ current in mammalian cardiac myocytes (Sanguinetti and Jurkiewicz, 1990). It is possible that the Cl^- channel in smooth muscle cells has Ca^{2+}-dependent and delayed rectification. It has also been suggested that the Cl^- channels have no intrinsic inactivation (Pacaud et al., 1992) and that the decay of spontaneous inward current may represent Cl^- channel closure after the decrease in $[Ca^{2+}]_i$ (Hogg et al., 1993).

Fourthly, the activation of $I_{Cl\text{-}Ca}$ may possibly be mediated by enzyme reaction which depends upon the elevation of $[Ca^{2+}]_i$, whereas that of $I_{K\text{-}Ca}$ is directly regulated by $[Ca^{2+}]_i$. Single channel current of small Ca^{2+}-dependent Cl^- channel in smooth muscle cells shows run-down in excised patch configuration (Klöckner, 1993; Renterghem and Lazdunski, 1993), suggesting the contribution of intracellular substance to the channel activity. Loirand et al. (1990) have shown that GTPγS activates $I_{Cl\text{-}Ca}$ by the elevation of $[Ca^{2+}]_i$ which is induced by the production of inositol 1,4,5-trisphosphate ($InsP_3$) via phospholipase C activation and that phorbol-12,13-dibutyrate (PDB), GDPβS or arachidonic acid does not elicit $I_{Cl\text{-}Ca}$ in cultured smooth muscle cells of the rat portal vein. In airway epithelia and cardiac myocytes, Cl^- channels are regulated by phosphorylation via cAMP-dependent protein kinase (McCann and Welsh, 1990; Bahinski et al., 1989). In guinea-pig tracheal smooth muscle cells, application of 10 μM isoproterenol, 1 mM 8-bromo-cAMP or 20 μM forskolin did not elicit $I_{Cl\text{-}Ca}$ at the holding potential of −60 mV when the pipette solution contained mainly 140 mM CsCl (Henmi et al., 1995). It is, therefore, unlikely that the activation of $I_{Cl\text{-}Ca}$ involves protein kinase A- or C-dependent pathways. The possible contribution of other signal transduction pathway was not further examined in the present study.

Major membrane currents elicited by agonists in tracheal smooth muscle cells are $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ which are due to Ca^{2+} release from storage sites, whereas non-selective cationic current is also activated by acetylcholine (Janssen and Sims, 1992). Since the reversal potential of Cl^- current is assumed to be between −18 and −47 mV in smooth muscles (Aickin and Vermuë, 1983; Gerstheimer et al., 1987), $I_{Cl\text{-}Ca}$ is inward current around resting membrane potential of −60 mV and may contribute to membrane depolarization. Small membrane depolarization in comparison with large contraction, which has been defined as pharmaco-mechanical coupling (Coburn and Yamaguchi, 1977; Farley and Miles, 1977), might be attributable to the counter balance of simultaneous activation of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$. This possibility, however, may not be real based upon the different time courses of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ shown in this study. Another feature of agonist-induced changes in membrane potential in tracheal smooth muscle is the generation of slow wave. Moreover, spontaneous slow waves have been observed in some preparations of trachea or bronchi (for a review, see Kirkpatrick, 1980). Although random occurrence of spontaneous outward and inward currents in single cells cannot explain

slow wave in a tissue preparation at this moment, the physiological significance of the different time courses of $I_{K\text{-}Ca}$ and $I_{Cl\text{-}Ca}$ may possibly be cyclical changes in inward and outward current conductance underlying the slow wave.

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References

Aickin, C.C. and N.A. Vermuë, 1983, Microelectrode measurement of intracellular chloride activity in smooth muscle cells of guinea-pig ureter, Pflügers Arch. 397, 25.

Akbarali, H.I. and W.R. Giles, 1993, Ca^{2+} and Ca^{2+}-activated Cl^- currents in rabbit oesophageal smooth muscle, J. Physiol. (London) 460, 117.

Amédéé, T., C.D. Benham, T.B. Bolton, G. Byrne and W.A. Large, 1990, Potassium, chloride and non-selective cation conductances opened by noradrenaline in rabbit ear artery cells, J. Physiol. (London) 423, 551.

Bahinski, A., A.C. Nairn, P. Greengard and D.C. Gadsby, 1989, Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes, Nature 340, 718.

Benham, C.D. and T.B. Bolton, 1986, Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit, J. Physiol. (London) 381, 385.

Bolton, T.B. and D.J. Beech, 1992, Smooth muscle potassium channels: their electrophysiology and function, in: Potassium Channel Modulators, eds. A.H. Weston and T.C. Hamilton (Blackwell Scientific Publication, London) p. 144.

Byrne, N.G. and W.A. Large, 1988, Membrane ionic mechanisms activated by noradrenaline in cells isolated from the rabbit portal vein, J. Physiol. (London) 404, 557.

Coburn, R.F. and T. Yamaguchi, 1977, Membrane potential-dependent and -independent tension in the canine tracheal muscle, J. Pharmacol. Exp. Ther. 201, 276.

Farley, J.M. and P.R. Miles, 1977, Role of depolarization in acetylcholine induced contractions of dog trachealis muscle, J. Pharmacol. Exp. Ther. 201, 199.

Gerstheimer, F.P., M. Muhleisen, D. Nehring and V.A.W. Kreye, 1987, A chloride bicarbonate exchanging anion carrier in vascular smooth muscle, Pflügers Arch. 409, 60.

Hamill, O.P., A. Marty, E. Neher, E. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membranes patches, Pflügers Arch. 391, 85.

Henmi, S., Y. Imaizumi, K. Muraki and M. Watanabe, 1995, Characteristics of caffeine-induced and spontaneous inward currents and related intracellular Ca^{2+} storage sites in guinea-pig tracheal smooth muscle cells, Eur. J. Pharmacol. 282, 219.

Hisada, T., Y. Kurachi and T. Sugimoto, 1990, Properties of membrane currents in isolated smooth muscle cells from guinea-pig trachea, Pflügers Arch. 416, 151.

Hogg, R.C., Q. Wang and W.A. Large, 1993, Time course of spontaneous calcium-activated chloride currents in smooth muscle cells from the rabbit portal vein, J. Physiol. (London) 464, 15.

Horn, R. and A. Marty, 1988, Muscarinic activation of ionic currents measured by a new whole-cell recording method, J. Gen. Physiol. 92, 145.

Imaizumi, Y. and M. Watanabe, 1981, The effect of tetraethylammonium chloride on potassium permeability in the smooth muscle cell membrane of canine trachea, J. Physiol. (London) 316, 22.

Imaizumi, Y., K. Muraki and M. Watanabe, 1990, Characteristics of transient outward currents in smooth muscle cells from the ureter of the guinea-pig, J. Physiol. (London) 427, 301.

Inoue, R. and S. Chen, 1993, Physiology of muscarinic receptor-operated nonselective cation channels in guinea-pig ileal smooth muscle, in: Nonselective Cation Channels: Pharmacology, Physiology and Biophysics, eds. D. Siemen and J. Nescheler (Birkhäuser Verlag, Basel) p. 261.

Janssen, L.J. and S.M. Sims, 1992, Acetylcholine activates non-selective cation and chloride conductances in canine and guinea-pig tracheal myocytes, J. Physiol. (London) 453, 197.

Janssen, L.J. and S.M. Sims, 1993, Histamine activates Cl^- and K^+ currents in guinea-pig tracheal myocytes: convergence with muscarinic signaling pathway, J. Physiol. (London) 465, 661.

Janssen, L.J. and S.M. Sims, 1994, Spontaneous transient inward currents and rhythmicity in canine and guinea-pig tracheal smooth muscle cells, Pflügers Arch. 427, 473.

Kirkpatrick, C.T., 1975, Excitation and contraction in bovine tracheal smooth muscle, J. Physiol. (London) 244, 263.

Kirkpatrick, C.T., 1980, Tracheobronchial smooth muscle, in: Smooth Muscle: an Assessment of Current Knowledge, eds. E. Bülbring, A.F. Brading, A.W. Jones and T. Tomita (Edward Arnold, London) p. 385.

Klöckner, U., 1993, Intracellular calcium ions activate a low-conductance chloride channel in smooth-muscle cells isolated from human mesenteric artery, Pflügers Arch. 424, 231.

Klöckner, U. and G. Isenberg, 1991, Endothelin depolarizes myocytes from porcine coronary and human mesenteric arteries through a Ca-activated chloride current, Pflügers Arch. 418, 168.

Lamb, F.S., K.A. Volk and E.F. Shibata, 1994, Calcium-activated chloride current in rabbit coronary artery myocytes, Circ. Res. 75, 742.

Loirand, G., P. Pacaud, C. Mironneau and J. Mironneau, 1990, GTP-binding proteins mediate noradrenaline effects on calcium and chloride currents in rat portal vein myocytes, J. Physiol. (London) 428, 517.

Markwardt, F. and G. Isenberg, 1992, Gating of maxi K^+ channels studied by Ca^{2+} concentration jumps in excised inside-out multichannel patches (myocytes from guinea pig urinary bladder), J. Gen. Physiol. 99, 84.

Marty, A., Y.P. Tan and A. Trautman, 1984, Three types of calcium-dependent channels in rat lacrimal glands, J. Physiol. (London) 357, 297.

Mayer, M.L., 1985, A calcium-activated chloride current generates the after-depolarization of rat sensory neurons in culture, J. Physiol. (London) 364, 217.

McCann, J.D. and M.J. Welsh, 1990, Regulation of Cl^- and K^+ channels in airway epithelium, Annu. Rev. Physiol. 52, 115.

Muraki, K., Y. Imaizumi, T. Kojima, T. Kawai and M. Watanabe, 1990, Effects of tetraethylammonium and 4-aminopyridine on outward currents and excitability in canine tracheal smooth muscle cells, Br. J. Pharmacol. 100, 507.

Muraki, K., Y. Imaizumi and M. Watanabe, 1992, Ca-dependent K channels in smooth muscle cells permeabilized by β-escin recorded using the cell-attached patch-clamp technique, Pflügers Arch. 420, 461.

Ohta, T., S. Ito and Y. Nakazato, 1993, Chloride currents activated by caffeine in rat intestinal smooth muscle cells, J. Physiol. (London) 465, 149.

Ohya, Y., K. Kitamura and H. Kuriyama, 1987, Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell, Am. J. Physiol. 252, C401.

Pacaud, P., G. Loirand, G. Gregoire, C. Mironneau and J. Mironneau, 1992, Calcium-dependence of the calcium-activated chloride current in smooth muscle cells of rat portal vein, Pflügers Arch. 421, 125.

Pacaud, P., G. Loirand, J.L. Lavie, C. Mironneau and J. Mironneau, 1989a, Calcium-activated chloride current in rat vascular smooth muscle cells in short-term primary culture, Pflügers Arch. 413, 629.

Pacaud, P., G. Loirand, C. Mironneau and J. Mironneau, 1989b, Noradrenaline activates a calcium-activated chloride conductance and increases the voltage-dependent calcium current in cultured single cells of rat portal vein, Br. J. Pharmacol. 97,139.

Renterghem, C.V. and M. Lazdunski, 1993, Endothelin and vasopressin activate low conductance chloride channels in aortic smooth muscle cells, Pflügers Arch. 425, 156.

Sanguinetti, M.C. and N.K. Jurkiewicz, 1990, Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class III antiarrhythmic agents, J. Gen. Physiol. 96, 195.

Small, R.C., 1982, Electrical slow waves and tone of guinea-pig isolated trachealis muscle: effects of drugs and temperature changes, Br. J. Pharmacol. 77, 45.

Stephens, N.L. and E. Kroeger, 1970, Effect of hypoxia on airway smooth muscle mechanics and electrophysiology, J. Appl. Physiol. 28, 630.

Suzuki, M., K. Muraki, Y. Imaizumi and M. Watanabe, 1992, Cyclopiazonic acid, an inhibitor of the sarcoplasmic reticulum Ca^{2+}-pump, reduces Ca^{2+}-dependent K^+ currents in guinea-pig smooth muscle cells, Br. J. Pharmacol. 107, 134.

Wang, Q., R.C. Hogg and W.A. Large, 1992, Properties of spontaneous transient inward currents recorded in smooth muscle cells isolated from the rabbit portal vein, J. Physiol. (London) 451, 525.

White, C.R., T.S. Elton, R.L. Shoemaker and T.A. Brock, 1995, Calcium-sensitive chloride channels in vascular smooth muscle cells, Proc. Soc. Exp. Biol. Med. 208, 255.