Effects of Excess K⁺ on Carbachol-induced Contractions in the Guinea-Pig Tracheal Muscle

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Abstract

1. In smooth muscles isolated from the guinea-pig trachea, the effects of dihydropyridines, nifedipine and nicardipine on contractions produced by carbachol (Cch) were studied in normal (6 mM) and excess K⁺ concentration (60 mM). The tonic contraction produced by 1 μM Cch was highly dependent on the external Ca²⁺ concentration ([Ca²⁺]₀) and was not significantly affected by cyclopiazonic acid or thapsigargin, Ca²⁺ uptake inhibitor.

2. [Ca²⁺]₀-tension curves were steeper in the presence of 1 μM Cch (the Hill coefficient: 2.5) than in the presence of 60 mM K⁺ (Hill coefficient: 1.6) and their ED₅₀ of Ca²⁺ was 0.16 and 0.39 mM, respectively. An increase of K⁺ to 60 mM in the presence of 1 μM Cch shifted the curve to the left roughly in parallel (ED₅₀: 0.12 mM, Hill coefficient: 2.3).

3. [Ca²⁺]₀-tension curve in the presence of 1 μM Cch was shifted to the right in parallel by nifedipine (1 μM). This was markedly potentiated by 60 mM K⁺ (the increase in ED₅₀ of Ca²⁺ being 3 times at 6 mM and 15 times at 60 mM K⁺). No tension was evoked by Ca²⁺ up to 2.5 mM in 60 mM K⁺ solution containing 1 μM nifedipine but no Cch.

4. In the absence of nifedipine, Cch-induced contractions were potentiated by 60 mM K⁺, whereas in the presence of nifedipine, Cch-induced contractions were markedly inhibited by 60 mM K⁺. These mechanical changes were accompanied by an increase or a decrease in intracellular Ca²⁺.

5. A hypothesis is presented to explain the results which suggests that the kinetics of Ca²⁺ influx though a single type of pathway is modulated by membrane potential and receptor activation and that the susceptibility of the pathway to dihydropyridine blockade is closely related to the Ca²⁺ influx kinetics with receptor activation reducing and membrane depolarization increasing the susceptibility.

Key words: dihydropyridines, Ca channel, trachea, receptor-mediated contraction, carbachol

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Introduction

It is generally assumed in smooth muscles, including airway muscles that there are separate $\text{Ca}^{2+}$ influx pathways: one voltage-dependent and the other receptor-mediated. The voltage-dependent influx pathway is dependent on L-type $\text{Ca}^{2+}$ channels which can be blocked by dihydropyridines, such as nifedipine as has been shown in airway muscle cells with the patch-clamp method (Hisada et al., 1990; Kotlikoff, 1988; Marthan et al., 1989; Worley and Kotlikoff, 1990). Since contractions produced by several agonists, such as carbachol (Cch), are often only weakly susceptible to dihydropyridines, it is possible that a receptor-activated pathway, which does not involve L-type $\text{Ca}^{2+}$ channels is responsible for $\text{Ca}^{2+}$ influx (Ahmed et al., 1985; Bolton, 1979; Coburn, 1979). Although evidence for the existence of such a pathway has come from experiments which measured the intracellular concentration of calcium ions ([Ca$^{2+}$]$_i$) in cultured airway muscle cells using fura-2 signals (Murray et al., 1993; Murray and Kotlikoff, 1991), the properties of this pathway have not yet been characterized in airway muscles.

The susceptibility of agonist-induced contractions to $\text{Ca}^{2+}$-channel blockers varies depending on the agonist concentration (Farley and Miles, 1978; Parekh and Brading, 1991), the external concentration of $\text{Ca}^{2+}$ ([Ca$^{2+}$]$_o$), and the type of smooth muscle (Croxton et al., 1994). This could result from different contributions of the voltage-dependent and receptor-mediated $\text{Ca}^{2+}$ influx pathways as well as from differences in the relative contribution of $\text{Ca}^{2+}$ release from intracellular stores.

In previous experiments, the properties of a receptor-mediated $\text{Ca}^{2+}$ influx pathway in the guinea-pig tracheal muscle were studied by comparing the effects of verapamil on the relationships between [Ca$^{2+}$]$_o$ and tension obtained in the presence of Cch with those obtained in high concentrations of K$^+$ (Gonda et al., 1988). These experiments showed that the dissociation constant of verapamil was nearly the same when the contractions were produced by either excess K$^+$ or Cch, suggesting that a common $\text{Ca}^{2+}$ influx pathway was responsible for these contractions. The decreased susceptibility of the contractions produced by Cch to verapamil was suggested to result from an improved $\text{Ca}^{2+}$-contraction coupling, as indicated by a decrease in the EC$_{50}$ and an increase in the Hill coefficient of the [Ca$^{2+}$]$_o$-tension curve, i.e., an increase in the cooperativity of $\text{Ca}^{2+}$ interaction with the pathway.

In the present experiments several factors which might affect the blocking activity of L-type $\text{Ca}^{2+}$ channel blockers have been further studied using Cch-induced contractions of the tracheal smooth muscles isolated from the guinea-pigs. Excess K$^+$ was found to significantly increase the susceptibility of Cch-induced contractions to dihydropyridines and no clear evidence was found for the existence of two independent, dihydropyridine-sensitive and insensitive $\text{Ca}^{2+}$ influx pathways when Cch in a concentration of 1 $\mu$M was applied. These observations can be explained by the hypothesis that the affinity of $\text{Ca}^{2+}$ influx pathway to dihydropyridine-channel blockers is decreased when muscarinic receptors are activated with higher concentrations of Cch whereas the affinity is increased by membrane depolarization.
Methods

The methods were essentially similar to those previously described (Ito et al., 1995). Guinea-pigs (250–350 g) were stunned by a blow to the head and killed by exsanguination. The trachea was dissected out, connective tissue was removed from the dorsal surface, and the trachea was opened longitudinally along the central surface. Four preparations each containing only one cartilage ring were excised from a central region of the trachea after carefully removing the mucosa with fine forceps under a binocular microscope. The cartilage was cut off leaving about 1.5 mm at each end of the muscle, one side was connected to a strain gauge transducer by a fine silk thread and the other side was fixed to the bottom of a chamber (0.2 ml) using a fine pin. The chamber was perfused with physiological solution, prewarmed to 35°C, at a constant rate of 1 ml min⁻¹. The control solution contained (mM): NaCl 127, KHCO₃ 6, CaCl₂ 2.4, MgCl₂ 1.2, glucose 12, HEPES buffer 10 (pH adjusted to 7.4 at 35°C with NaOH). When the external concentration of K⁺ concentration ([K⁺]₀) was increased or Ca²⁺ was omitted, the osmolarity was kept constant by altering the concentration of NaCl. EGTA (1 mM) was always added to the Ca²⁺-free solution, but EGTA was removed 5 min before changing [Ca²⁺]₀.

In experiments where mechanical responses were measured, four muscle strips from each animal were used for simultaneous recording. The preparations were stretched by applying tension of about 2 mN and allowed to stabilize at least for 1 hr before starting experiments. During the stabilization period, contractures produced by increasing [K⁺]₀ to 60 mM for 10 min were recorded twice. Indomethacin (1 μM) was added to all solutions to block a contribution of endogenous prostaglandins; all experiments were carried out in the dark to minimize photolysis of nifedipine.

Changes in [Ca²⁺]ᵢ were estimated by measuring the ratio of fluorescence emitted at 510 nm from fura-2 loaded preparations when excited alternatively with light of wavelengths 340 and 380 nm (see Ito et al., 1995). Preparations, similar to those used in the mechanical experiments, were mounted in a chamber (0.2 ml) and viewed with an inverted microscope (Diaphoto TMD 300, Nikon, Japan) connected to a Ca²⁺ imager (Argus-50/CA, Hamamatsu Photonics, Japan). These preparations were loaded with fura-2 AM (10 μM) for 2 hrs at room temperature (24°C) and measurements were started after superfusing the preparation with physiological solution, again prewarmed to 35°C, for 20–30 min at a constant rate of 1 ml min⁻¹.

All drugs used were obtained from Sigma (St Louis, USA). Nifedipine and nicardipine were dissolved freshly every 2–3 days in ethanol to make stock solutions, 1 mM. The Hill coefficient of [Ca²⁺]₀-tension curves and dissociation constant (Kₐ) of the dihydropyridines were obtained as described previously (Gonda et al., 1988). Each [Ca²⁺]₀-tension curve was constructed from the mean of the data based on 8–12 preparations. All numerical data are expressed as means±S.E.M.
Results

Cch-induced contractions

In the presence of 2.4 mM Ca\textsuperscript{2+}, the addition of 1 µM Cch produced a sustained contraction, termed a Cch-induced contraction, which was quickly abolished by removing external Ca\textsuperscript{2+} even in the continuous presence of Cch (Fig. 1A). When 2.4 mM Ca\textsuperscript{2+} added to a tissue bathed in Ca\textsuperscript{2+}-free physiological saline containing 1 µM Cch it produced a tonic contraction, termed a Ca\textsuperscript{2+}-induced contraction, which was very similar to the Cch-induced contraction. The time to reach 50% maximum tension was 68±12 sec for Cch-induced and 97±16 sec for Ca\textsuperscript{2+}-induced contraction (n=12). When 1 µM Cch was applied after removal of Ca\textsuperscript{2+} a small transient contraction was observed if Cch was applied within 2–3 min of Ca\textsuperscript{2+} removal; if Ca\textsuperscript{2+} was removed for longer than 4 min, no such contraction was produced.

The similarity of Cch-induced and Ca\textsuperscript{2+}-induced contractions, the quick relaxation by Ca\textsuperscript{2+} removal, and only a small transient Cch-induced contraction shortly after Ca\textsuperscript{2+} removal suggest that Ca\textsuperscript{2+} influx is mainly responsible for these contractions. To confirm this possibility, the effect of inhibitors of Ca\textsuperscript{2+} pump in the sarcoplasmic reticulum (SR), cyclopiazonic acid (CPA) or thapsigargin (TPG) on these contractions were studied. Fig. 1B shows a typical example of the effect of 5 µM CPA. CPA and TPG increased muscle tone very slowly, but the rate of increase greatly varied in different preparations (Takemoto et al., 1998), but CPA had no significant effect on the contractions. TPG also failed to alter the contractions significantly (not shown). The muscle tone increased by CPA itself recovered taking more than 30 min on wash-out whereas no recovery of the TPG-induced contraction was observed in 30 min.

Ca\textsuperscript{2+}-induced contraction in the presence of 60 mM K\textsuperscript{+}, 1 µM Cch, and 60 mM K\textsuperscript{+} + 1 µM Cch

Ca\textsuperscript{2+}-induced contractions were produced by the cumulative application of Ca\textsuperscript{2+} in the presence of 60 mM K\textsuperscript{+}, 1 µM Cch, and 60 mM K\textsuperscript{+} + 1 µM Cch.

![Fig. 1. Contraction produced by carbachol (Cch, 1 µM) in guinea-pig tracheal muscle and effects of Ca\textsuperscript{2+} removal before (A) and after application of 5 µM cyclopiazonic acid (CPA). EGTA (1 mM) was added to Ca\textsuperscript{2+}-free solutions but it was removed about 5 min before Ca\textsuperscript{2+} (2.4 mM) reapplication in the presence of Cch. CPA was applied 15 min before Cch application in B and it was present throughout.](image-url)
Channel blockers on receptor-mediated contraction

Fig. 2. Ca\(^{2+}\) concentration-tension curve in the presence of 1 \(\mu\)M Cch (circle, averaged from 16 preparations), 60 mM K\(^{+}\) (square, \(n=8\)), and 1 \(\mu\)M Cch + 60 mM K\(^{+}\) (triangle, \(n=8\)). Data represent the means of values and S.E.M. shown by vertical bars. The maximum tension obtained in the presence of 1 \(\mu\)M Cch was taken as 100%.

Effects of nifedipine and nicardipine on Cch-induced contraction

Fig. 3 shows effects of 1 \(\mu\)M nifedipine and nicardipine on Cch-induced contraction curves determined in control solution (2.4 mM Ca\(^{2+}\), 6 mM K\(^{+}\)). Both nifedipine and nicardipine shifted the curve to the right and slightly increased its slope. Both antagonists markedly reduced the contractions produced by low concentrations of Cch but had little effect on the contractions produced by Cch concentrations higher than 10 \(\mu\)M. Similar effects have been reported for canine tracheal muscle (Farley and Miles, 1978) and guinea-pig gastric muscle (Parekh and Brading, 1991). At a concentration of 1 \(\mu\)M nifedipine or nicardipine completely abolished contractions evoked by 60 mM K\(^{+}\). The block with nifedipine in the presence of 0.6 mM Ca\(^{2+}\) is shown in Fig. 6B.

Effects of nifedipine and nicardipine on \([\text{Ca}^{2+}]_o\)-tension curve

The effects of nifedipine and nicardipine on Ca\(^{2+}\)-induced contractions, determined in the
presence of 1 μM Cch were also studied. As shown in Fig. 4, the effects were essentially similar to those on Cch-tension curve (Fig. 3), although the inhibitory effect of nicardipine at higher [Ca]₀ was stronger than nifedipine. Nifedipine (1 μM) increased the ED₅₀ of Ca²⁺ from 0.16 to 0.45±0.07 mM (n=8).

**Effects of nifedipine and nicardipine on [Ca²⁺]₀-induced curve in the presence of 60 mM K⁺**

As shown in Fig. 5, [Ca²⁺]₀-tension curve obtained in solutions containing both 1 μM Cch and 60 mM K⁺ was shifted markedly to the right by nifedipine (1 μM), ED₅₀ of Ca²⁺ being increased to 1.8±0.19 mM (n=8) from 0.12 mM in the presence of 1 μM Cch and 6 mM K⁺. In the presence of 1 μM Cch the pKa value of nifedipine was increased from 7.2 to 8.8 and that of nicardipine from 7.0 to 8.0 by increasing [K⁺]₀ from 6 to 60 mM K⁺.
Channel blockers on receptor-mediated contraction

Fig. 5. \([\text{Ca}^{2+}]_o\)-tension curve before (Control, circle, \(n=16\)) after application of 1 \(\mu\)M nifedipine (square, \(n=8\)) or 1 \(\mu\)M nicardipine (triangle, \(n=8\)) in the presence of 60 mM K\(^+\) + 1 \(\mu\)M Cch. The control curve was the same as that obtained in the presence of 1 \(\mu\)M Cch + 60 mM K\(^+\) in Fig. 2.

Contractions produced by a combination of 60 mM K\(^+\) and 1 \(\mu\)M Cch in the absence and presence of nifedipine

Fig. 6 shows a typical record of contractions produced by a combination of 1 \(\mu\)M Cch and 60 mM K\(^+\) before (A) and after application of 1 \(\mu\)M nifedipine (B). Since the effects of nifedipine were stronger with lower concentrations of Ca\(^{2+}\) (Figs. 4 and 5), the experiment shown in Fig. 6 was carried out in the presence of 0.6 mM Ca\(^{2+}\). As expected from Fig. 2, a contraction produced by 60 mM K\(^+\) was increased by an addition of 1 \(\mu\)M Cch and similarly, a contraction produced by 1 \(\mu\)M Cch was increased by increasing [K\(^+\)]\(_o\) from 6 to 60 mM K\(^+\) (Fig.

Fig. 6. Typical records of the effect of nifedipine on contractions produced by 60 mM K\(^+\), 1 \(\mu\)M Cch, and 60 mM K\(^+\) + 1 \(\mu\)M Cch in the presence of 0.6 mM Ca\(^{2+}\). A: in the absence and B: in the presence of 1 \(\mu\)M nifedipine which was applied 15 min before application of 60 mM K\(^+\).
6A). In the presence of 1 μM nifedipine no contraction was produced by 60 mM K+, and only a small contraction was evoked by adding 1 μM Cch. In the presence of 1 μM nifedipine, the Cch–induced contraction was slightly smaller at 6 mM K+ and markedly inhibited by increasing [K+]o to 60 mM (Fig. 6B).

**Effects of 60 mM K+ on Cch–induced contraction and [Ca2+]i in the absence and presence of nicardipine**

The changes in [Ca2+]i, measured with fura-2 (Fig. 7, lower) together with the associated mechanical responses (Fig. 7, upper trace) were determined also at 0.6 mM [Ca2+]o when 1 μM Cch was added initially at 6 mM K+ and then when [K+]o was increased to 60 mM in the presence of 1 μM Cch (Fig. 7A). This sequence was repeated after adding 1 μM nicardipine (Fig. 7B). Due to its photosensitivity effects of nifedipine were not examined in the [Ca2+]i measurements. It can be seen that in the absence of nicardipine, an increase in [K+]o potentiated both the rise in [Ca2+]i and the amplitude of the associated Cch–induced contraction. In contrast, in the presence of nicardipine, a similar rise in [K+]o reduced both the elevated [Ca2+]i, and contraction that had been produced by Cch. The same results were confirmed in 3 other preparations.

**Discussion**

It has been reported that in cat tracheal muscle Ca2+ influx from the external medium is mainly involved in K+-contractures whereas the release of Ca2+ from intracellular stores plays an important role in contractions induced by acetylcholine (10 μM) (Ito and Itoh, 1984). However our experiments in guinea-pig tracheal muscle have shown that removal of external Ca2+ rapidly inhibited the tonic contractions produced by 1 μM Cch. Moreover, neither cyclopiazonic acid nor thapsigargin, agents which inhibit Ca2+ uptake into the intracellular store, significantly changed contractions produced by 1 μM Cch. These results suggest that in

![Fig. 7. The potentiation and the inhibition of Cch-induced contraction (upper) and intracellular Ca2+ concentration measured with fura-2 (lower trace) produced with 60 mM K+ before (A) and after application of 1 μM nicardipine. Nicardipine was applied 10 min before 2nd application of Cch. Fura-2 signals are expressed as the ratio of fluorescence excited at 340 and 380 nm.](image)
guinea-pig tracheal muscle Ca\textsuperscript{2+} influx is mainly responsible for Cch-induced contraction. With higher concentrations of Cch, however, the release of Ca\textsuperscript{2+} from stores may contribute to the contraction significantly.

When the ED\textsubscript{50} of Ca\textsuperscript{2+} was determined for Cch-induced and K\textsuperscript{+}-induced contractions it was found to be lower for Cch than for K\textsuperscript{+}. Moreover the Hill coefficient was larger for Cch-induced contractions than for those evoked by 60 mM K\textsuperscript{+}. At first sight these results suggest that different Ca\textsuperscript{2+} influx pathways are involved in these contractions; a receptor-mediated pathway for the former and the L-type Ca\textsuperscript{2+} channel for the latter. As might be expected the contractions produced by increasing [K\textsuperscript{+}]\textsubscript{o} were readily abolished by the L-type Ca\textsuperscript{2+} channel blockers nifedipine or nicardipine. However, both these blockers also reduced the responses produced by low and moderate concentrations of Cch suggesting that a common channel was involved. The Cch-induced tension curve was shifted to right more or less in parallel by nifedipine and nicardipine. These findings suggest that L-type Ca\textsuperscript{2+} channels might be partly responsible for triggering contraction, when concentrations of Cch up to 1 \mu M are applied. The nifedipine- and nicardipine-resistant component of Cch-induced contraction triggered by higher concentrations of Cch could have resulted from Ca\textsuperscript{2+} influx through a distinct receptor-mediated pathway.

The ability of either nicardipine or nifedipine to block Cch-induced contractions was markedly potentiated when the membranes of the cells were depolarized by the addition of 60 mM K\textsuperscript{+}. This potentiation could be explained by a decrease in driving force for Ca\textsuperscript{2+} influx or if the receptor-mediated pathway was inhibited by membrane depolarization, or if the receptor-mediated pathway had become more susceptible to the dihydropyridines when the cells were depolarized. The voltage dependent nature of dihydropyridine block of L-type Ca\textsuperscript{2+} channels has been described previously (Nelson et al., 1989), but it seems unlikely that such an effect would contribute to our observations. The membrane potential of bronchial smooth muscle is about −50 mV in control solutions (Ahmed et al., 1985; Honda et al., 1986), and about −40 mV in the presence of 1 \mu M Cch (unpublished observations), a potential well positive of those which make dihydropyridines inactive.

In cultured cells from human tracheobronchial muscle, in the absence of a Ca\textsuperscript{2+} channel blocker, the increase in [Ca\textsuperscript{2+}]\textsubscript{i} induced by histamine is decreased either when [K\textsuperscript{+}]\textsubscript{o} is increased (Murray and Kotlikoff, 1991) or when the cell is depolarized electrically (Murray et al., 1993). This has been explained by a decrease in driving force for Ca\textsuperscript{2+} influx (the Ca\textsuperscript{2+} equilibrium potential, E\textsubscript{ca}−the membrane potential, V\textsubscript{m}). Although the reversibility of the inhibitory effect of excess K\textsuperscript{+} is most easily explained by this idea, it is doubtful if this could be applied to the present experiments in guinea-pig tracheal muscle. In the absence of the blockers, Cch-induced contractions and increases in [Ca\textsuperscript{2+}]\textsubscript{i} were potentiated by increasing K\textsuperscript{+} to 60 mM; these contractions and increases in [Ca\textsuperscript{2+}]\textsubscript{i} were clearly larger than those induced by 60 mM K\textsuperscript{+} alone (Figs. 6, 7). Since the driving force must have been decreased by increasing [K\textsuperscript{+}]\textsubscript{o} the increase in both [Ca\textsuperscript{2+}]\textsubscript{i} and tension must have resulted from facilitated Ca\textsuperscript{2+} influx through the receptor-mediated pathway or to facilitated Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channel as a result of muscarinic stimulation. We favour the latter possibility since this facilitation was markedly suppressed by the dihydropyridine nicardipine. Thus the
significance of the change in driving force on responses in control solution is not clear. In the presence of nicardipine when Cch-induced contraction was greatly inhibited by 60 mM K⁺, the driving force for Ca²⁺ will be little changed. Firstly [Ca²⁺], decreased so causing the E_{Ca} (RT/2F ln [Ca²⁺]₀/[Ca²⁺]) to increase, e.g. from +90 mV to +104 mV (assuming a decrease of [Ca²⁺], from 600 nM to 200 nM at 0.6 mM [Ca²⁺], by 60 mM K⁺ in the presence of nicardipine). Secondly an increase in [K⁺]₀ from 6 to 60 mM in the presence of 1 μM Cch might be expected to decrease the V_m from −40 to around −20 mV. Thus the decrease in the driving force will only be from 130 mV to 124 mV. Clearly such a small change is unlikely to explain a large relaxation produced by 60 mM K⁺ when nifedipine or nicardipine is present (Figs. 6, 7). The most likely explanation for the inhibition of Cch-induced contraction by 60 mM K⁺ is that when dihydropyridines are bound to the Ca²⁺ pathway, its Ca²⁺ permeability is decreased by membrane depolarization or that the susceptibility of the receptor-mediated pathway to dihydropyridines is increased by membrane depolarization.

In summary, no clear evidence was obtained for two separate Ca²⁺ influx pathways in the present experiments. The most likely explanation for our finding is that the kinetics of Ca²⁺ influx through the single pathway is modulated by receptor activation and also by membrane potential, and that the susceptibility of the pathway to dihydropyridines is closely related to the Ca²⁺ influx kinetics, as suggested by Gonda et al. (1988). The susceptibility of this pathway to dihydropyridine blockade is reduced depending on the degree of receptor activation while it is increased by membrane depolarization.

References

Ahmed, F, Foster, R.W., and Small, R.C. (1985). Some effects of nifedipine in guinea-pig isolated trachealis. Br. J. Pharmacol. 84, 861–869.

Bolton, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59, 607–718.

Coburn, R.F. (1979). Electromechanical coupling in canine trachealis muscle: acetylcholine contractions. Am. J. Physiol. 236, C177–184.

Croxton, T.L., Fleming, C. and Hirshman, C.A. (1994). Expression of dihydropyridine resistance differs in porcine brachial and tracheal smooth muscle. Am. J. Physiol. 267, L106–112.

Farley, J.M. and Miles, P.R. (1978). The sources of calcium for acetylcholine-induced contractions of dog tracheal smooth muscle. J. Pharmacol. Exp. Ther. 207, 340–346.

Gonda, H., Baba, K., Satake, T., Takagi, K. and Tomita, T. (1988). The dissociation constant of verapamil estimated from its effect on Ca concentration–tension curves in guinea-pig tachael muscle. Pul Pharmacol. 1, 7–13.

Hisada, T., Kurachi, Y. and Sugimoto, T. (1990). Properties of membrane currents in isolated smooth muscle cells from guinea–pig trachea. Pflügers Arch. 416, 151–161.

Honda, K., Satake, T., Takagi, K. and Tomita, T. (1986). Effects of relaxants on electrical and mechanical activities in the guinea-pig tracheal muscle. Br. J. Pharmacol. 87, 665–671.

Ito, Y. and Itoh, T. (1984). The roles of stored calcium in contractions of cat tracheal smooth muscle produced by electrical stimulation, acetylcholine and high K⁺. Br. J. Pharmacol. 83, 667–676.

Ito, Y., Takagi, K. and Tomita, T. (1995). Relaxant actions of isoprenaline on guinea–pig isolated tracheal smooth muscle. Br. J. Pharmacol. 116, 2738–2742.

Kotlikoff, M.I. (1988) Calcium currents in isolated canine airway smooth muscle. Am. J. Physiol.
Marthan, R., Martin, C., Amedee, T. and Mironneau, C. (1989). Calcium channel currents in isolated smooth muscle cells from human bronchus. *J. Appl. Physiol.* **66**, 1706-1714.

Murray, R.K., Fleischmann, B.K. and Kotlikoff, M.I. (1993). Receptor-activated Ca influx in human airway smooth muscle: use of Ca imaging and perforated patch-clamp techniques. *Am. J. Physiol.* **264**, C485-490.

Murray, R.K. and Kotlikoff, M.I. (1991). Receptor-activated calcium influx in human airway smooth muscle cells. *J. Physiol.* **45**, 123-144.

Nelson, M.T. and Worley, J.F. (1989). Dihydropyridine inhibition of single calcium channels and contraction in rabbit mesenteric artery depends on voltage. *J. Physiol.* **412**, 65-91.

Parekh, A.B. and Brading, A.F. (1991). The sources of calcium for carbachol-induced contraction in the circular muscle of guinea-pig stomach. *Br. J. Pharmacol.* **104**, 412-418.

Takemoto, M., Takagi, K., Ogino, K. and Tomita, T. (1998). Comparison of contractions produced by carbachol, thapsigargin and cyclopiazonic acid in the guinea-pig tracheal muscle. *Br. J. Pharmacol.* **124**, 1449-1454.

Worley, J.F. and Kotlikoff, M.I. (1990). Dihydropyridine-sensitive single calcium channels in airway smooth muscle cells. *Am. J. Physiol.* **259**, L468-480.

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