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THE INTERNAL CALCIUM STORE IN AIRWAY MUSCLE: EMPTYING, REFILLING AND CHLORIDE

POSSIBLE NEW DIRECTIONS FOR DRUG DEVELOPMENT

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Abstract—This review examines the ionic mechanisms underlying acetylcholine (Ach) depolarization of airway smooth muscle and suggests that multiple mechanisms are involved. Increased chloride and non-specific cation conductance, and decreased or rapidly inactivating potassium conductances seem to be involved. Chloride ions also seem to play an important role in determining whether Ca^{2+} remains inside or is replenished in the sarcoplasmic reticulum (SR). The physiological role of Ach-induced depolarization is analysed and is suggested to be the promotion of the refilling of Ca^{2+} stores, partly through a direct refilling of SR-Ca^{2+} stores by way of an L-type Ca^{2+} channel. This refilling is promoted by Ca^{2+} channel agonists and is independent of the transmembrane potential. Ca^{2+}-release by a variety of agonists leads to depolarization and stable membrane oscillations which depend on the action of the Ca^{2+}-store uptake mechanisms in order to function. These oscillations may play a role in prolonged bronchoconstriction. Better knowledge of the control mechanisms of Ca_i^{2+} is likely to reveal new targets for the therapy of asthma and provide a better understanding of the function of airway smooth muscle.

Airway smooth muscle contractions in response to agonists utilize, in many cases, internal Ca^{2+} stores [1, 2]. For example, acetylcholine (Ach†)-induced responses in canine tracheal [2, 3] and bronchial [4] muscle strips are not reduced markedly by L-Ca^{2+} channel antagonists, by hyperpolarization by cromakalin sufficient to hold the voltage well below threshold for L-Ca^{2+} channels (VOCC) [3, 5] or even by removal of external Ca^{2+} [1, 2]. In the last case, the initial contraction is of normal size but is not maintained and after inducing two or three responses Ach is no longer effective. This suggests that after contraction the intracellular Ca^{2+} stores must be refilled by Ca^{2+} entering from the extracellular space [2].

One major puzzle has concerned the role, if any, of Ach-induced depolarization. This depolarization is at maximum about 20–25 mV from a membrane potential of −55–60 mV and is limited by outward rectification [1, 3, 6, 7] but is stable for many minutes (1–3, 5–7). As a result of the outward rectification Ca^{2+}-spikes from the opening of VOCC do not normally occur and it is unclear whether Ca^{2+} enters through these channels or by some other means to refill the internal Ca^{2+} stores. The Ach-induced depolarization *per se* does not contribute to contractile force since brief repolarization of strips of trachea in the double sucrose gap fails to affect contractile responses [1, 2]. The ionic mechanisms of the Ach depolarization are also unknown. It is known that in tracheal strips studied in the double sucrose gap the conductance changes associated with the depolarization are very small when measured after repolarization by current passing. Also, the extrapolated (necessary since depolarization is limited by outward rectification) reversal potential is about 25 mV positive to the resting potential [2, 7]. Furthermore, a number of K^+-channel blockers including charybdotoxin, Ba^{2+} and glibenclamide, fail to reduce while TEA increases the amplitude of the Ach depolarization when measured from the original membrane potential [7]. TEA at high concentrations does, however, slow the initial rate of Ach depolarization. These results, plus the well-demonstrated existence of large Ca^{2+}-activated K^+-channels in airway smooth muscle [8, 9], suggest that the occurrence of peristent Ach depolarization requires the existence of a mechanism of rapid deactivation of the K^+-channels opened by elevation of Ca^{2+} or by depolarization. If this were not so then there would be a notable conductance increase and K^+-channel blockade would potentiate depolarization markedly. With the double sucrose gap technique it is not possible to observe an early, rapidly decaying, conductance increase so a brief initial period of increased conductance following Ach administration would be missed.

We have recently attempted to seek answers to two questions derived from the above information: (1) what is the mechanism of the Ach depolarization? (2) What is the physiological role of the Ach depolarization?

Mechanism of the Ach depolarization

The answers to this first question are incomplete. Double sucrose gap studies at 27° suggest that chloride channels are present in canine tracheal smooth muscle and that they are likely to be open at the resting potential. Table 1 summarizes the

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† Abbreviations: Ach, acetylcholine; VOCC, voltage operated Ca^{2+} channels; IP_3, 1,4,5 inositol triphosphate; EGTA, ethylineglycol-bis (β-aminoethyl ether)-N,N,N,N'tetracetic acid; TEA, tetraethylammonium ion; CPA, cyclopiazonic acid; SR, sarcoplasmic reticulum; 4,AP, 4-aminopyridine; EJP, excitatory junction potential.

Table 1. Evidence for chloride channels in canine airway smooth muscle

	ΔV_m (mV)	Electrostatic potential (% change)	Outward rectification	Effect of DIDS
Isethionate substitution	+10–20	+30–40	Reduced	Depolarization to isethionate substitution reduced by 50%
Nitrate substitution	0–−3	0–−10	Not reduced	Not studied

See Ref. 5.

Table 2. Evidence that chloride channels might be involved in Ach depolarization in canine airway

Effects of	EJP* Duration of Cl-free (min)	Ach* depolarization (% change)	V_{REV}‡ (Δ mV)	Contraction† Basal tension (% change)	Contraction† Ach-induced (10^{-6} M) tension (% change)
Isethionate substitution	5–10	+19	Not done	Not done	Not done
	30	−40–50	−10–15	+10–15	−40
	60–90	−85	Voltage insensitive	+10–15	−40–60
Nitrate substitution	5–30	−20–40	−10–15	+10–25	−10–25
	60–90	−400	−10–15	+20–30	−20–30

At 27° in the double sucrose gap note that the reversal potential (V_{REV}) was about 30 mV positive to the resting potential (V_m) for Ach depolarization and was shifted negatively by substitution of isethionate or nitrate for chloride (10–15 mV).

* At 27°; double sucrose gap.

† At 37°; muscle bath; comparisons are percentage of Ach contractions.

‡ See Ref. 5.

findings providing supporting evidence of the existence of chloride channels which may contribute to outward rectification. Their existence has been more definitively confirmed recently by cell attached patch clamp studies [Janssen L and Sims S, unpublished] of single canine tracheal smooth muscle cells.

The question of participation of chloride channels opening in Ach depolarization has turned out to be difficult to answer with conventional electrophysiological techniques. As summarized in Table 2, EJPs and Ach depolarizations were initially increased compared to values predicted from the current voltage relationship, and were then reduced after 30 min when either isethionate (a non-penetrating anion and not a substrate for HCO_3^-–Cl^- exchange in other biological systems) or nitrate (a rapidly penetrating anion and a substrate for HCO_3^-–Cl^- exchange in other biological systems) was substituted for chloride [10–14]. Moreover, in some experiments isethionate substitution not only increased initially the EJPs compared to expected values from the associated depolarization, but also chloride restoration after 30 min decreased them before returning them to control values. These results would be expected if the chloride gradient was a determinant of Ach depolarization amplitudes. Moreover, the contractile responses were reduced in these experiments. After prolonged (60–90 min) exposure to isethionate-substituted Kreb's solution, the EJP became very small and voltage-independent (no change in amplitude with de- or hyperpolarization) and contractions disappeared. The fact that the extrapolated reversal potential shifted to a more negative (10–15 mV) value implies the presence of other Ach-induced channel events besides chloride channel opening, e.g. K^+-channel closure by Ach would contribute more prominently to depolarization after loss of the chloride gradient. However, the persistence of a net positive value for this potential in the presumed absence of any chloride gradient also indicates the involvement of other ions with an equilibrium potential positive to the resting potential. The observations with isethionate substitution are consistent with, but do not establish, chloride channels being opened by Ach.

The effects of the substitution of nitrate for chloride were more difficult to fit into this picture of chloride channel opening by Ach. Nitrate penetrates many [10] but not all [10, 11] chloride channels rapidly and is a good substrate for HCO_3^-–Cl^- exchange [12, 13]. Thus, following the hypothesis that Cl^- channel opening contributes prominently to Ach depolarization, it was surprising that nitrate substitution reduced rapidly the amplitude of EJPs and Ach depolarizations, and also shifted the extrapolated reversal potential to a more negative value.

An explanation of this paradox may be given partially by experiments in which contractions of tracheal strips at 37° were measured (Table 2). The substitution of chloride with either nitrate or isethionate caused a partial tonic contraction, and a reduction in the contraction to 10^{-6} M Ach, with isethionate substitution being more effective. Restoration of chloride rapidly relaxed the tonic contractions and restored responses to Ach. In the case of isethionate substitution, the sum of the tonic contractions and the Ach contraction was always less than the control Ach response whereas in the case of nitrate substitution this sum was similar in magnitude to the control Ach contraction.

When these experiments were repeated with the removal of Ca^{2+} (Ca^{2+}-free + 0.1 mM EGTA) at the same time as the substitution of chloride, the contraction in chloride-free solutions was reduced markedly. Moreover, the contractile responses to Ach exposure repeated every 20 min fell off rapidly and the first one in isethionate-substituted media was significantly smaller than in the comparable control containing chloride but no Ca^{2+}. Restoration of chloride but not Ca^{2+} rapidly relaxed any tonic contraction but did not restore responses to Ach. Restoration of Ca^{2+} but not chloride caused further tonic contractions. In the case of isethionate substitution, Ca^{2+} restitution did not restore Ach-induced contractions while restoration of chloride allowed recovery of Ach contractile responses. In the case of nitrate-substituted medium, Ca^{2+} restoration partially restored the Ach response, the sum of the tonic and Ach-induced contractions being approximately equal to the control Ach response. Nifedipine did not abolish the tonic contractions in chloride-free medium and did not reduce the amplitude of Ach contractions.

If contractile responses to Ach are taken to be primarily a measure of Ca_i^{2+} release in the circumstances of our study (see below), these studies suggest that Ca_i uptake and release depends on the presence of a diffusible anion in the cytoplasm in SR and that, when isethionate depletes internal chloride, the SR loses much of its releasable Ca^{2+}. Nitrate also fails to substitute for chloride in maintaining releasable Ca^{2+} in stores but the mechanism is unclear unless the anion channels in SR membrane, unlike those in the plasmalemma, do not admit nitrate freely. It is obvious that much more work needs to be done to support these interpretations but a failure in the control of the Ca^{2+} content of SR after 30 min or more in chloride-free medium could explain: (1) the fall-off in EJP and Ach depolarization (reduced Ca^{2+} release); (2) the negative shift of the reversal potential for Ach depolarization in chloride-free medium (submaximal Ca^{2+} release); (3) the loss of contractile responses to Ach in isethionate-substituted media; and (4) the difficulty in refilling Ca^{2+} stores in isethionate-substituted media.

These findings do not exclude the possibility that chloride outward currents play a role in Ach depolarization. Indeed, the time course of altered responses in isethionate solution is consistent with an early reduction of Ach depolarization related to effects on plasmalemmal channels and a later reduction related to loss of Ca^{2+} from the stores.

If this explanation of chloride ion involvement is correct, another Ca^{2+}-activated channel besides the chloride channel and with an equilibrium potential positive to the resting potential must also be involved in Ach depolarization. This follows from the residual depolarization after 30 min in chloride-free media with an extrapolated reversal potential positive to the resting potential. The most likely candidate is a non-specific cation channel through which Na^+ or Ca^{2+} could enter causing depolarization. We have found no direct evidence for such a channel; neither removal of Na^+ or removal of Ca^{2+} affected Ach depolarization. However, such studies with conventional electrophysiological techniques are inconclusive because of rapidly shifting and uncontrolled ionic gradients. Patch clamp studies [Janssen L and Sims S, unpublished observations] now in progress should resolve this matter.

Physiological role of the Ach depolarization

Ach depolarization, like depolarization by other agonists which operate through IP_3-triggered Ca_i release, does not appear to contribute directly to contraction. Recent data [3] suggest that it, together with a transmembrane voltage-independent process, operates to enhance refilling of the Ca_i^{2+} stores when they are emptied by agonist stimulation. Figure 1 shows the result of giving repeated Ach (3×10^{-6} M) challenges to strips of canine trachealis muscle in the presence or absence of 2×10^{-6} M nifedipine or 10^{-8} M Bay K 8644. Bay K 8644 did not enhance the contractile responses to Ach which were remarkably stable over time in the control and in the presence of the L-Ca^{2+} channel opener. However, when nifedipine was continually present the contractile responses after the first two stimuli began to decline and fell to a plateau at about 65% of the initial value.

From this experiment we may make the paradoxical conclusion that Ca^{2+} entry through L-Ca^{2+} channels either does not normally play a role in maintaining Ca_i^{2+} stores (as Bay K 8644 has no effect) or that it does (as nifedipine reduces the response). The paradox can be resolved by the suggestion that Bay K 8644 has no effect because these channels are already opened by acetylcholine and that nifedipine closes them gradually, thus, revealing the partial dependence of the Ca^{2+} stores on the entrance of Ca^{2+} from outside the cell. The ability of the contractile response to be maintained at a submaximal level (80% of control) in the presence of nitrendipine reflects either Ca^{2+} entry through another type of Ca^{2+} channel or an efficient recycling of Ca^{2+} into the stores.

If the amount of Ca^{2+} in the stores after the ninth Ach test contraction is assessed (Fig. 2) by exposure of the tissues to Ca^{2+}-free (+100 μM EGTA) solution before the 10th addition of Ach, the internal Ca^{2+} stores in nifedipine-treated tissues are found to be depleted of Ca^{2+} compared to controls. The question then arises as to how the Ca^{2+}, which normally enters through L-Ca^{2+} channels, gets into the store: directly or after uptake from the cytoplasm?

First, it should be noted that the depolarization

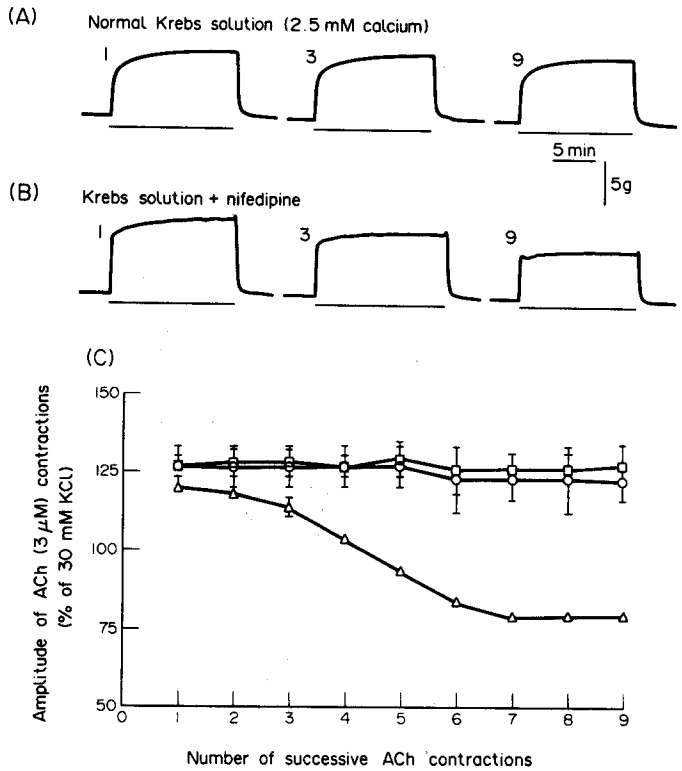

Fig. 1. Effect of 2 μM nifedipine and 10 nM BAY K 8644 on contractions induced by repetitive Ach stimulation in normal Krebs solution in canine tracheal smooth muscle. (A) Typical examples of the muscle response to the 1st, 3rd and 9th consecutive Ach (3 μM) stimulations in a normal (2.5 mM Ca^{2+}) Krebs medium. Successive stimulations were separated by a 15-min interval. Bars represent Ach present in the medium. (B) Conditions identical to A but the medium contained 2 μM nifedipine. (C) A summary of the amplitude of the Ach contraction as a function of the number of successive Ach stimulations. (○) Control; (□) +10 nM BAY K 8644; (△) +2 μM nifedipine. Mean ± SEM of four experiments.

initiated by acetylcholine is to −30–−35 mV, a level at which VOC channel openings are likely to be increased but to nowhere near the maximum [14, 15]. Kotlikoff [15] showed that isolated canine tracheal muscle cells had a voltage-operated Ca^{2+} current which was activated at membrane potentials depolarized to more than −45 mV and by Bay 8644 but was inhibited by dihydropyridine VOCC antagonists at concentrations higher than those required in heart muscle cells. For our purposes we will consider these channels to be of the L-type. Whether or not Ach increases the Ca^{2+} current through these channels is unclear. If this Ca^{2+} enters the cell through L-Ca^{2+} channels such as those described by Kotlikoff [15] and is then pumped from the cytosol into the internal stores [3], agents which inhibit Ca^{2+} pump activity should eventually deplete the internal stores of releasable Ca^{2+}, thus, making refilling impossible.

In fact, with CPA [16] which inhibits the internal Ca^{2+} pump, and ryanodine, which makes Ca_i^{2+} stores leaky [17], it is possible to deplete Ach-releasable Ca^{2+} stores estimated as described above. Figure 3 shows the effects of CPA on such stores in canine trachealis [3]. However, the effects of CPA are not due to a failure of the contractile response to Ach. Indeed the response is of near normal amplitude if external Ca^{2+} is present but it becomes more dependent on Ca^{2+} entrance through L-Ca^{2+} channels (Fig. 4) than when the Ca^{2+} stores are full. L-Ca channel blockers inhibit contractions by 60% compared with control values. When Ca^{2+} release channels in SR were made leaky by ryanodine [18], an increase in basal tension occurred associated with a decrease in Ach-releasable Ca_i^{2+}. This basal tension increase was blocked by nifedipine and enhanced markedly by Bay K 8644 [4].

After the refilling of Ach-releasable Ca_i^{2+} stores from the cytoplasm has been inhibited by CPA a process unaccompanied by membrane potential depolarization, the store can be refilled from the extracellular space by exposing the tissue to Bay K 8644 (Fig. 5). Moreover, this process of Bay K 8644-accelerated Ca^{2+} entrance into its electrochemical gradient is unaffected by the membrane potential of the cell. Depolarization of 10–20 mV by tetraethylammonium ions (20 mM) or by hyperpolarization by 12–20 mV by chromakalim (3×10^{-6} M) did not change this effect of Bay K 8644 [19]. From these results we conclude that the Ca^{2+} channels which allow direct refilling of Ca^{2+} stores are unusual in that they are blocked

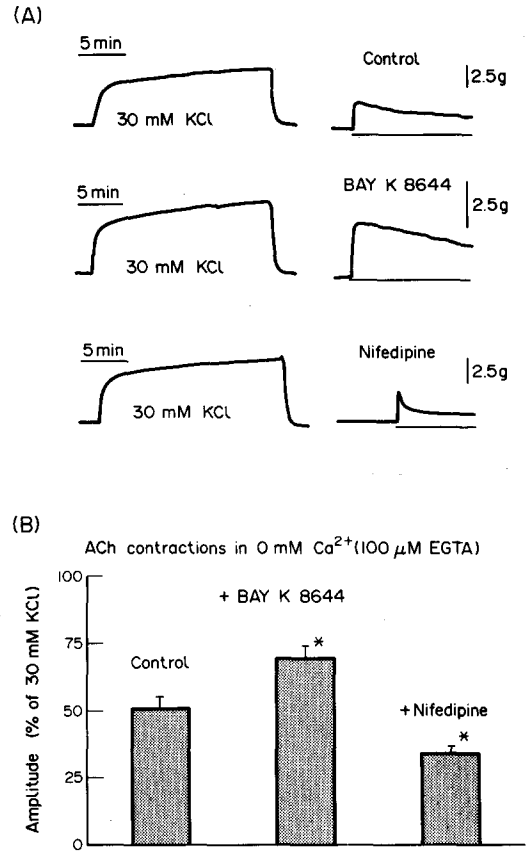

Fig. 2. Ach-induced contraction in a Ca^{2+}-free medium: influence of nifedipine and BAY K 8644. After successive Ach stimulation (cf. Fig. 1) preparations were washed in a Ca^{2+}-free medium containing 100 μM EGTA for 10 min and then stimulated with 3 μM Ach. (A) Typical examples of the transient Ach contractions in Ca^{2+}-free medium (right tracings). Amplitude is compared with that of the high potassium-induced contraction in a Ca^{2+}-containing medium in the same tissue (left tracings). Note the marked increase in the size of the transient contraction when the previous successive Ach stimulations have been performed in a Krebs solution containing 10 nM BAY K 8644 (middle right trace) and the decreased size of the transient contraction when 2 μM nifedipine were present (lower right trace), compared to control (upper right trace). (B) The amplitude of Ach contraction in Ca^{2+}-free medium has been plotted as a function of the muscle response to a stimulation with a 30 mM KCl-containing medium (mean ± SEM, N = 4). * Significantly different ($P < 0.05$) from control.

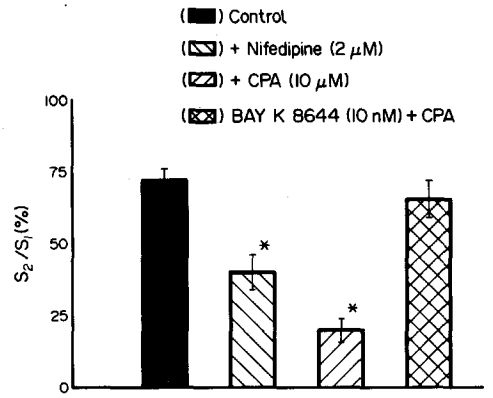

Fig. 3. Effect of 2 μM nifedipine, 10 mM BAY K 8644 and 10 μM CPA on active refilling of Ach-sensitive intracellular Ca^{2+} stores. Intracellular Ach-sensitive Ca^{2+} stores were depleted by repetitive stimulations of the preparation with 3 μM Ach in Ca^{2+}-free medium (100 μM EGTA). The first Ach contraction in Ca^{2+} medium is referred to as the original response (S_1). Preparations were then washed several times in Ca^{2+}-free medium. After the washes, 2 μM nifedipine, 10 μM CPA or 10 μM CPA and 10 nM BAY K 8644 were added 15 min before the refilling period of 20 min during which preparations were challenged with 3 μM Ach. The refilling medium contained 2.5 mM Ca^{2+}. Amplitudes of transient Ach contractions in Ca^{2+}-free medium after refilling (S_2) are expressed as percentages of the original Ach transient contraction before depletion (S_1). Mean ± SEM, N = 4.

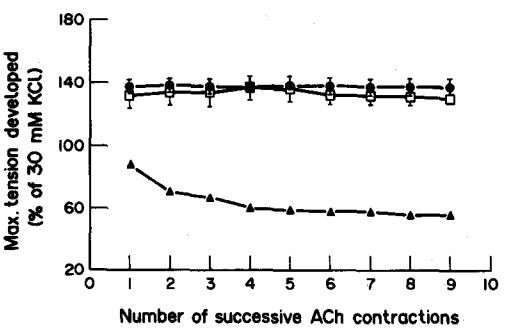

Fig. 4. Repetitive acetylcholine contractions in CPA containing Krebs solution; influence of nifedipine. The maximum amplitude of contraction in calcium-containing medium is plotted against the number of the ACh stimulation and is expressed as a percentage of 30 mM KCl-induced contraction in the same strip. (●) Control, N = 15; (□) CPA, N = 5; (▲) CPA + nifedipine, N = 6. Each point represents the mean ± SD of N experimental data.

by dihydropyridine antagonists and opened by dihydropyridine agonists but are insensitive to the transmembrane voltage. Their insensitivity to the membrane potential might be a result of either their special properties or their location at the entrance to superficial SR, so that they read only the potential difference between the SR interior and the extracellular space, or both of these possibilities.

These findings indicate first that active transport across the SR membrane normally plays a role in refilling Ca_i stores but than an alternate path from the extracellular space can be made available by opening L-Ca^{2+} channels in canine trachealis. Recent studies show that Ach, but not high K^+, can also open this path. The residual 40% of control Ach contractions in the presence of nifedipine and CPA may represent Ca^{2+} entering through receptor-operated channels opened by Ach or Ca^{2+} being recycled through another CPA-insensitive store.

The magnitude of the potential difference between

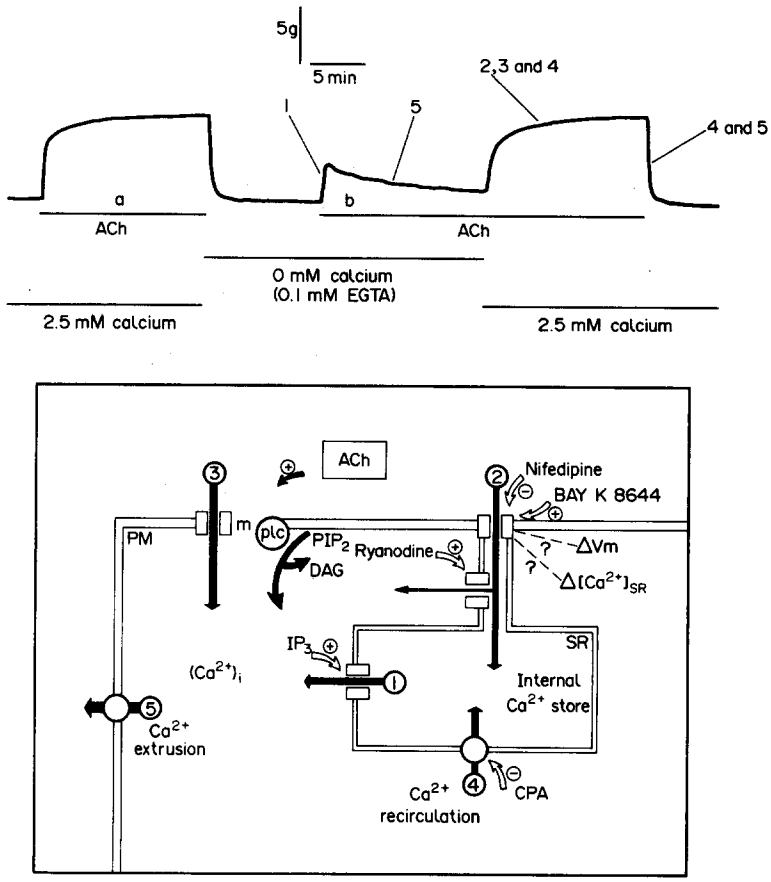

Fig. 5. Tentative model of E–C coupling in canine tracheal smooth muscle. Upon Ach stimulation, muscle contraction is associated with the recruitment of distinct Ca^{2+} pools resulting in an increase in cytosolic Ca^{2+} concentration. (1) Ca^{2+} is released from internal store(s), presumably in the SR, following stimulation of release channels by IP_3. This Ca^{2+} release from internal stores accounts for the initial component of Ach contraction (phasic component) in Ca^{2+}-containing medium (Tracing a) and for the transient Ach contraction recorded in Ca^{2+}-free medium (Tracing b). (2) Ach stimulation also, opens dihydropyridine-sensitive Ca^{2+} channels but not directly under the control of the plasmalemma (PM) potential allowing direct loading of the SR. Some Ca^{2+} entering the cell via this special pathway has access to the cytosol under regular conditions and, therefore, contributes though minimally (20–30%) to contraction. This Ca^{2+}, however, is helping restore the internal Ca^{2+} pool to its optimum capacity for release upon next agonist stimulation. When this special pathway is altered following partial opening of ryanodine-sensitive channels, Ca^{2+} leaks into the cytosol and induces a tonic contraction. (3) Opening of dihydropyridine-insensitive receptor-operated Ca^{2+} channels in the PM, induces an influx of extracellular Ca^{2+} into the cytosol which, under regular conditions, contribute 40–50% to the tonic part of Ach contraction. (4) Part (30–40%) of the nifedipine-insensitive tonic contraction results from a CPA-sensitive recirculation of cytosolic Ca^{2+} to the SR. (5) Upon cessation of Ach stimulation, cytosolic Ca^{2+} concentration decreases rapidly as part of the Ca^{2+} is taken up by the SR and part is extruded out of the cell, resulting in muscle relaxation. However, during prolonged Ach stimulation in Ca^{2+}-free medium (Tracing b), Ca^{2+} released from internal store(s) is extruded out of the cell with little recirculation into the SR, as indicated by the marked decrease of response to a second Ach stimulation in Ca^{2+} medium.

SR and the extracellular fluid is unknown. As noted above it may depend upon the functioning of chloride as a counter ion for Ca^{2+} and upon the extent of Ca^{2+} binding within the SR. Loss of Ca^{2+} from the SR might be exceeded by the loss of chloride ions during Ach stimulation, thus, leading to depolarization. The existence of Ca^{2+} channels directly connecting SR to the extracellular space has not yet been given ultrastructural support. Also, the existence of chloride channels in the smooth muscle SR has not been established but their existence seems to be a reasonable possibility as they do exist in skeletal and cardiac muscle [20, 21]. Our observations have led us to make a speculative model of control of intracellular Ca^{2+} in airway muscle (Fig. 6). In this model, we suggest that agonists like Ach act by producing IP_3 after phospholipase C activation, a process well established to occur in this tissue [22]. This causes Ca^{2+} release from SR. The release of Ca^{2+} from SR or an associated effect,

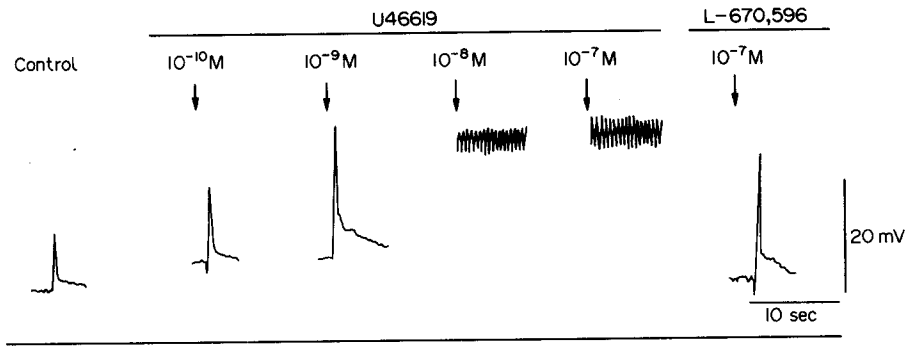

Fig. 6. Representative tracing showing the effect of U46619 (10^{-10}–10^{-7} M) on electrical responses to field stimulation (two pulses at 10 pulses/sec, 40 V) followed by the addition of the TxA_2 receptor antagonist L-670,596. Resting membrane potential prior to control response was −59 mV. U46619 at 10^{-10} and 10^{-9} M concentrations potentiated the amplitude and duration of the field stimulation EJP with slight depolarization of the membrane potential. Concentrations of U46619 at 10^{-8} M or greater caused depolarization of the membrane leading to oscillations in the membrane potential. The excitatory effects of U46619 were established after incubation in the tissue for 10 min. Increasing incubation times with L-670,596 (10^{-7} M) still resulted in an EJP with a potentiated amplitude. The tissues were pretreated with 10^{-6} M indomethacin.

such as depolarization across a Ca^{2+} channel connecting SR and the extracellular space, leads to Ca^{2+} entry, via its electrochemical gradient, into SR; this is independent of a Ca^{2+} pump process. Also involved is a Ca^{2+} recycling process or entrance of Ca^{2+} not involving L-Ca^{2+} channels but requiring uptake into SR via the Ca^{2+} pump. This must be postulated to account for the ongoing contractions in response to Ach in the presence of dihydropyridine antagonists and for the action of CPA or ryanodine eliminating this component of the response. Ach depolarization may also increase Ca^{2+} influx via non-L-type Ca^{2+} channels not associated with SR. The proposed answer to the question as to what is the physiological role of Ach depolarization is, therefore, to promote Ca^{2+} entrance into cytoplasm through receptor-operated channels and to open other L-type Ca^{2+} channels which lead directly to the SR. So far, it is unclear whether or not Ach does so by depolarization of the membrane between the SR interior and the extracellular fluid.

Initiation of slow waves by agonists causing Ca_i *release*

In canine 3rd to 4th order bronchi the action of agonists to release Ca_i^{2+} is similar to that in tracheal muscle. Also, both types of muscle, but bronchi more prominently, produce ongoing and regular oscillations of membrane potentials when the depolarizations associated with Ca_i release reach about 35–40 mV, a depolarization of about 15–20 mV. Figure 6 shows an example with the TxA_2 analogue U46619 and Table 3 summarizes the results with compounds which produce this phenomenon. It should be noted that TEA in high concentrations and Ba^{2+} which block several types of K^+-channel also produce this response. 4-AP also does so but primarily through Ach release. This and the failure of some other K^+ channel blockers to inhibit these slow waves speak against the possibility that Ca^{2+}-induced K^+ channel opening accounts for the repolarization component of each slow wave. L-Ca^{2+} channel blockers eliminate the slow waves but not the underlying depolarizations [4] and similar slow waves occurring spontaneously in guinea pig trachea are also abolished by L-Ca^{2+} channel blockers [23]. Substitution of Ca^{2+} with Sr^{2+}, which is taken up into SR less efficiently than Ca^{2+} [24, 25], reduces slow wave frequencies [4]. The fact that a variety of agents acting through different receptor mechanisms to depolarize airway muscle by mobilizing Ca_i release cause a very similar phenomenon, namely slow waves, being abolished by blocking of L-Ca^{2+} channels and not being susceptible to K^+-channel blockade, suggests that the slow waves are driven by the Ca^{2+} release from stores, a release which appears to trigger Ca^{2+} entry as discussed above. This Ca^{2+} entry could explain the depolarization phase of the slow wave and its sensitivity to dihydropyridines. The repolarization phase is unlikely to be due to opening of Ca^{2+}-activated K^+ channels in view of its insensitivity to their blockade (Table 3). A mechanism involving desensitization of Ca^{2+} entry or Ca^{2+} release caused by local elevation of Ca^{2+} beyond a certain point has been postulated [4, 26] but other possibilities including variations in IP_3 release controlled by Ca_i [27], or in interacting but separate Ca^{2+} stores of which one manifests Ca^{2+}-induced Ca^{2+} release [28–30] cannot be excluded. The possible role of simultaneous Ca_i, membrane potential and tension oscillations in airway hyperresponsiveness has not been explored.

Possibilities for new drug development

The fact that airway smooth muscle seems to have a mechanism for direct refilling of Ca_i^{2+} stores independent of transmembrane potential makes hyperpolarization *per se* an efficient way to inhibit bronchoconstriction or airway hyperresponsiveness. Only if hyperpolarization affects this direct pathway in addition to conventional pathways of Ca^{2+}

Table 3. Effects of various depolarizing agents on electrical activity of canine bronchial smooth muscle

Pharmacological agent	Membrane depolarization (mV)	Membrane oscillations		Effect of 10^{-7} M nitrendipine
		Amplitude (mV)	Frequency (Hz)	
Cch (3×10^{-3} M)	17.2 ± 2.0	4.1 ± 1.5	0.90 ± 0.17	No oscillation
U46619	21.5 ± 1.1	3.9 ± 0.9	2.35 ± 0.14	No oscillation
LTC_4 (10^{-7} M)	12.8 ± 2.2	4.8 ± 1.1	1.33 ± 0.14	Not tested
LTD_4 (10^{-7} M)	11.8 ± 2.6	1.8 ± 0.1	1.5 ± 0.10	Not tested
TEA (25 mM)	17.4 ± 0.6	13.1 ± 0.8	0.65 ± 0.03	No oscillation
4-AP (5 mM)	16.3 ± 0.7	9.0 ± 1.5	0.45 ± 0.04	No oscillation
Ba^{2+} (1 mM)	22.3 ± 3.1	3.3 ± 1.3	0.43 ± 0.05	No oscillation
TEA (25 mM) SR^{2+} (2.4 mM) Ca^{2+} (0 mM)	24.8 ± 1.8	14.0 ± 1.1	0.12 ± 0.07	No oscillation

Indomethacin 10^{-5} M was present in all cases.

Depolarization after exposure to phentolamine 10^{-7} M, propranolol 10^{-7} M and atropine 10^{-8} M was 12.9 ± 1.0 mV, amplitude of oscillations 6.3 ± 3.3 mV and frequency 0.12 ± 0.07 Hz for TEA and 4.0 ± 2.6 mV depolarization with no oscillations for 4-AP.

Values are means ± SE; N = 5–16.

influx will hyperpolarization inhibit airways muscle contraction effectively. Airway smooth muscle responses to most agonists will depend on the amount of Ca_i^{2+} in the stores. We are currently evaluating the possibility that airway hyperresponsiveness may result, in part, from altered Ca^{2+} content and release from internal stores. Even if this possible mechanism is not very important for airway hyperresponsiveness, pharmacological control of Ca^{2+} stores in airway muscle may become a very important tool to control bronchoconstriction. Possible sites of pharmacological control of SR Ca^{2+} release include inhibition of the internal Ca^{2+} pump, control of IP_3 receptor activation, control of Ca^{2+}-induced Ca^{2+} release, control of Ca^{2+} channels involved in direct refilling of Ca^{2+} stores and control of chloride mechanisms in SR Ca^{2+} uptake. It is difficult to predict which of these potential targets for drug action is more promising. If a Ca^{2+} channel associated with direct refilling of Ca^{2+} stores has special recognition properties for dihydropyridine and for other drugs acting on VOCC it would be a favourable target. Studies on the role of chloride in the refilling of Ca^{2+} stores might reveal a target for highly selective attack. In the case of the IP_3 binding site, molecular biology studies should soon reveal its similarity to or differences from sites in other cell types [31, 32]. The SR Ca^{2+} pump in airway muscle may also have special recognition properties; it will be necessary to establish its similarities to and differences from other internal Ca^{2+} pumps [33, 34] before it can become a target for control of internal Ca^{2+}.

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