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Some features of the spasmogenic actions of acetylcholine and histamine in guinea-pig isolated trachealis

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1 Intracellular electrophysiological recording showed that acetylcholine (1 μmol l^{-1}) and histamine (2 μmol l^{-1}) depolarized trachealis cells and often increased the frequency of slow waves. Higher concentrations of these agents caused greater depolarization and abolition of slow waves. Marked depolarization was often associated with the appearance of electrical 'noise'. These electrical phenomena were accompanied by tonic tension development in a contiguous segment of trachea.

2 Electrical 'noise' and tension evoked by high concentrations of acetylcholine or histamine could be dissipated by washing the agonist from the tissue. Acetylcholine-induced 'noise' was resistant to tetrodotoxin (3 μmol l^{-1}) and to hexamethonium (1 mmol l^{-1}).

3 Neither acetylcholine (10–1,000 μmol l^{-1}) nor histamine (2–200 μmol l^{-1}) increased the lanthanum-resistant calcium fraction of muscle-containing strips of trachea.

4 It is concluded that, while developing tension under the influence of acetylcholine or histamine, trachealis cells depolarize markedly but there is relatively little cellular influx of Ca^{2+}.

Introduction

There is evidence (Foster *et al.*, 1983a,b) that the spasmogenic effects of acetylcholine and histamine in guinea-pig trachealis represent the direct activation of muscle cell muscarinic and histamine H_1-receptors, respectively. However, the cellular events which follow the receptor activation in these two cases are poorly understood.

Recording extracellularly, Small (1982) showed that acetylcholine and histamine were each able to suppress spontaneous electrical slow waves of guinea-pig trachealis. Since slow wave discharge in airways smooth muscle is potential-dependent (Kirkpatrick, 1981), this suppression may reflect depolarizing actions of both acetylcholine and histamine. As far as we are aware, the action of acetylcholine on guinea-pig trachealis has not been studied by intracellular recording techniques, although histamine-induced depolarization has been reported (McCaig & Souhrada, 1980).

An ability of acetylcholine and histamine to depolarize guinea-pig trachealis might suggest that these agents should activate voltage-operated channels (Bolton, 1979) which admit Ca^{2+} into the smooth muscle cells. However, the resistance of acetylcholine- or histamine-induced tension development to inhibitors of Ca^{2+} influx (Cerrina *et al.*, 1983; Cheng & Townley, 1983; Foster *et al.*, 1984) suggests that Ca^{2+} entry through voltage-operated channels cannot be a major underlying mechanism. Acetylcholine might instead act to release Ca^{2+} from intracellular sites of sequestration (Creese & Denborough, 1981; Cerrina *et al.*, 1982).

In the present study we have recorded the intracellular electrical changes occurring during the actions of acetylcholine and histamine and have attempted to determine whether these spasmogens promote the cellular influx of Ca^{2+}.

Methods

Guinea-pigs (400–550 g) of either sex were killed by stunning and bleeding. Tracheae were excised from the animals, cleaned of adhering fat and connective tissue and opened by cutting longitudinally through the cartilaginous rings diametrically opposite the trachealis.

Intracellular recording

Simultaneous recording of intracellular electrical activity and the mechanical changes of a contiguous segment of trachea were performed as described by Dixon & Small (1983).

The effects of acetylcholine and histamine were examined by their addition to the Krebs solution superfusing the tissue. Attempts to construct cumulative concentration-effect curves for acetylcholine (1, 10, 100 and 1000 μmol l^{-1}) or histamine (2, 20 and 200 μmol l^{-1}) were made during the microelectrode

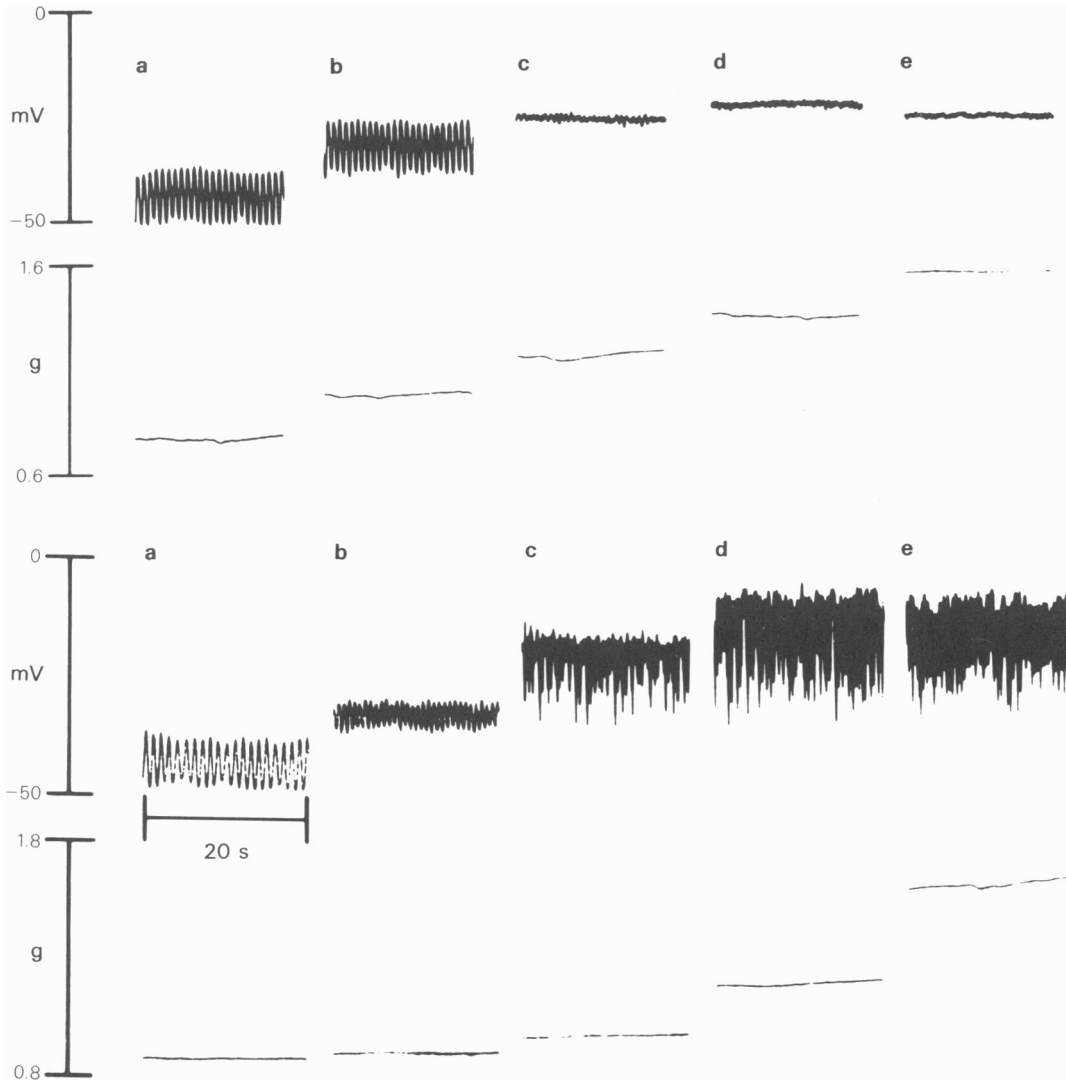

Figure 1 The effects of acetylcholine on the intracellular electrical activity and mechanical changes in two preparations of guinea-pig trachea. In each case the upper trace = membrane potential changes whilst the lower trace = tension changes of a contiguous segment of trachea. (a) Control, (b, c, d, e) 3 min after acetylcholine (1, 10, 100, 1000 μmol l^{-1}, respectively). In each preparation all electrical records are taken from a single cell. In the upper preparation, slow wave abolition was followed only by smooth depolarization. This became maximal before maximal tension development in the contiguous segment of trachea. In the lower preparation, acetylcholine (1 μmol l^{-1}) caused an increase in slow wave frequency. Note the electrical 'noise' evoked by higher concentrations of this agent. Onset of the 'noise' followed both the abolition of slow waves and a transient period of smooth depolarization (not shown).

impalement of a single cell. The contact time for each concentration of a particular agonist was such as to allow nearly full development of the tension rise attainable by that concentration of agonist. For acetylcholine this was 3 min and for histamine 6 min.

Measurement of the lanthanum-resistant calcium fraction

The method of Foster *et al.* (1983a) was used to measure the lanthanum-resistant calcium fraction of muscle-containing and muscle-free strips of trachea. Following tissue equilibration with MOPS physiological salt solution (Jetley & Weston, 1980) and with $^{45}Ca^{2+}$, test tissue strips were incubated in MOPS physiological salt solution with added acetylcholine (10,100 or 1000 μmol l^{-1}) or with added histamine (2, 20 or 200 μmol l^{-1}). Tissue strips acting as controls were treated with vehicle.

Three minutes (test and control tissues for acetylcholine) or 6 min (test and control tissues for histamine) later, tissues were removed from these media and placed in ice-cold oxygenated MOPS physiological salt solution containing $LaCl_3$ 10 mmol l^{-1}. Further processing of tissues, counting of radioactivity and calculation of the lanthanum-resistant calcium fraction were performed as previously described (Foster *et al.*, 1983a).

Drugs and solutions/statistical analysis of results

Drug concentrations are throughout expressed in terms of the base. The following drugs were used: acetylcholine chloride (BDH), hexamethonium bromide (Sigma), histamine acid phosphate (BDH), lanthanum chloride (BDH), tetrodotoxin (Sigma). Stock solutions of acetylcholine were prepared in absolute alcohol, those of other drugs in twice distilled water.

$^{45}Ca^{2+}$ was supplied as an aqueous solution of $CaCl_2$ by the Radiochemical Centre, Amersham. The specific activity of the material was 2 mCi 170 μg^{-1} Ca^{2+}.

A two-tailed,unpaired *t* test was used to assess the significance of differences between means.

Results

Intracellular electrophysiological recording

The electrical changes evoked by acetylcholine (1–1000 μmol l^{-1}) were complex and, to some extent, dependent on the type of spontaneous slow wave discharge exhibited by the cell under investigation.

In cells exhibiting periodic or continuous slow wave discharge of low amplitude (2–8 mV) acetylcholine (1 μmol l^{-1}) caused depolarization and an increase in slow wave amplitude and frequency. In cells exhibiting continuous discharge of slow waves of high (12–20 mV) amplitude, the same concentration evoked depolarization and an increase in slow wave frequency: however, the amplitude of the slow waves was reduced. Acetylcholine (10 μmol l^{-1}) evoked further depolarization (Figure 1 and Table 1) and a marked reduction in slow wave amplitude, as did acetylcholine (100 μmol l^{-1}) which usually abolished slow wave discharge. In some cells the suppression of slow waves was followed by a smoothly-developing depolarization but in the vast majority of cells, also by development of relatively high frequency and apparently random oscillations of potential, a few millivolts in amplitude (Figure 1). This type of activi-

Table 1 The effects of acetylcholine and histamine on the intracellular electrical activity of guinea-pig trachealis

		Apparent depolarization (mV)	*Maximal amplitude of slow waves* (mV)	*Slow wave frequency* (Hz)
Control		–	12.2 ± 1.1	1.00 ± 0.07
	1	8.3 ± 1.3	9.1 ± 1.4	1.20 ± 0.07
Acetylcholine	10	17.2 ± 1.9	3.0 ± 0.9	0.63 ± 0.20
(μmol l^{-1})	100	27.2 ± 2.6	0	0
	1000	31.4 ± 3.2	0	0
Control		–	10.6 ± 1.2	1.08 ± 0.08
	2	7.8 ± 1.1	7.2 ± 1.2	1.20 ± 0.08
Histamine	20	17.0 ± 1.1	1.3 ± 1.0	0.19 ± 0.13
(μmol l^{-1})	200	22.7 ± 2.5	0	0

The data represent the mean values from at least 7 (acetylcholine) or 6 (histamine) cells ± s.e. Each measurement was made 3 min (acetylcholine) or 6 min (histamine) after tissue exposure to the indicated concentration of agonist.

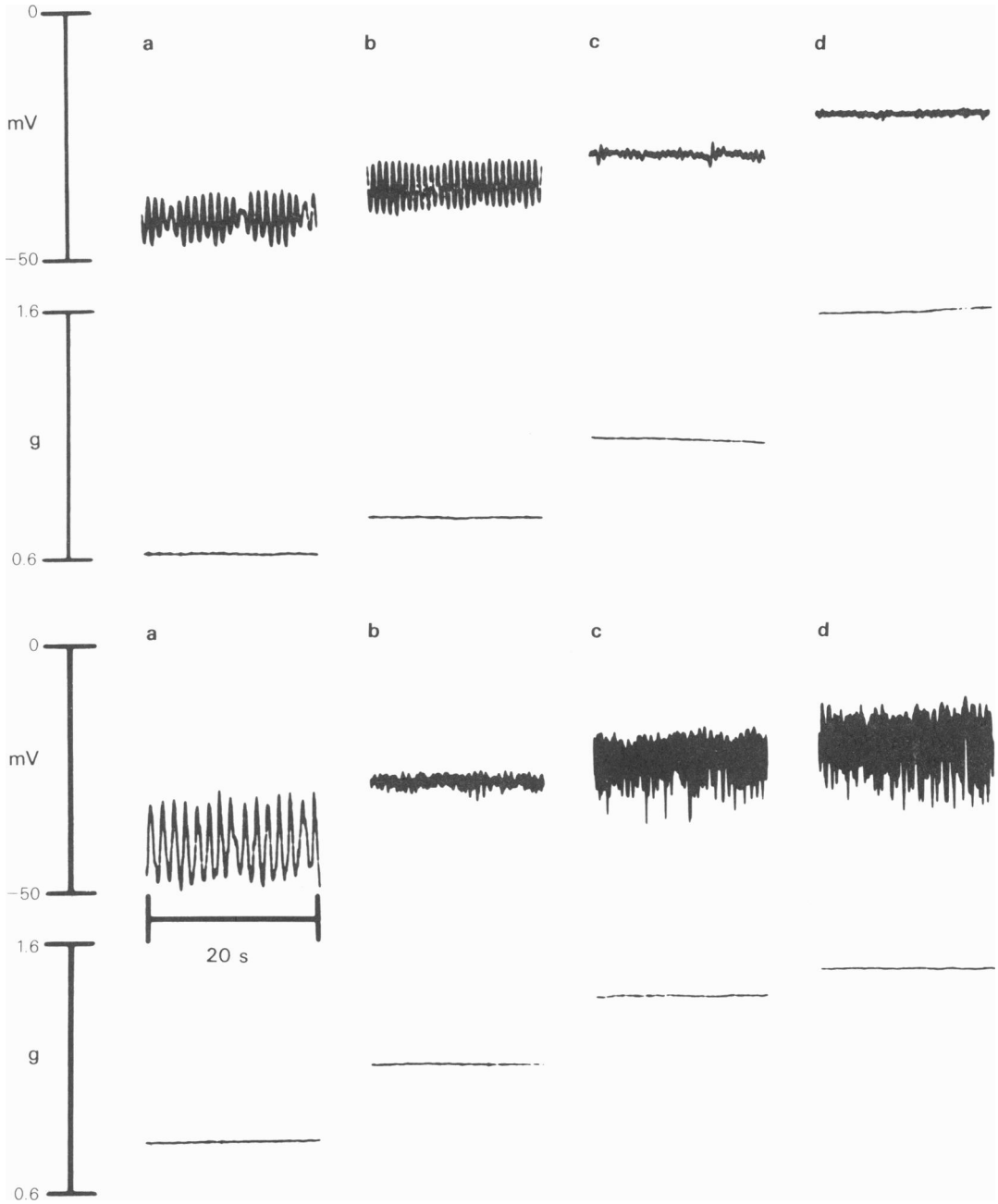

Figure 2 The effects of histamine on the intracellular electrical activity and mechanical changes in two preparations of guinea-pig trachea. In each case the upper trace = membrane potential changes whilst the lower trace = tension changes of a contiguous segment of trachea. (a) Control, (b, c, d) 6 min after histamine (2, 20, 200 μmol l^{-1}, respectively). In each preparation all electrical records are taken from the same cell. In the upper preparation, histamine (2 μmol l^{-1}) caused an increase in slow wave frequency. Higher concentrations of histamine abolished slow waves and yielded smooth depolarization. Note, in the lower preparation, the electrical 'noise' evoked by histamine (20 and 200 μmol l^{-1}).

ty often contained rapid deflections in the direction of hyperpolarization. The random oscillations (electrical 'noise') increased in amplitude as the concentration of acetylcholine was raised to 100 μmol l^{-1} (Figure 1).

Acetylcholine (1–1000 μmol l^{-1}) did not induce spike potentials but evoked concentration-dependent, tonic tension development by the contiguous segment of trachea. On returning to Krebs solution the tension was dissipated. Electrical 'noise', including the rapid hyperpolarizing deflections, was reduced as the cell slowly repolarized. When the cell had repolarized sufficiently, the discharge of slow waves often recommenced.

The electrical 'noise' evoked by acetylcholine was unaffected by either tetrodotoxin (3 μmol l^{-1}) (5 experiments) or hexamethonium (1 mmol l^{-1}) (3 experiments).

The electrical changes evoked by histamine (2–200 μmol l^{-1}) were similar to those evoked by acetylcholine. For example histamine (20 μmol l^{-1}) evoked depolarization (Figure 2 and Table 1) and suppressed slow wave activity. Further depolarization was induced by histamine (200 μmol l^{-1}), and sometimes (Figure 2) the cell membrane became electrically noisy and small, rapid hyperpolarizing deflections appeared. However, this type of activity was usually less marked than that observed with acetylcholine. Similarly, histamine caused concentration-dependent, tonic tension development by the contiguous segment of trachea.

Measurement of the lanthanum-resistant calcium fraction

Table 2 presents values of the lanthanum-resistant calcium fraction of control strips of trachea and of tissue strips treated with acetylcholine or histamine. Neither acetylcholine (10–1000 μmol l^{-1}) nor histamine (2–200 μmol l^{-1}) caused any significant change in the lanthanum-resistant calcium fraction.

The lanthanum-resistant calcium fraction of tissue taken from the tracheal wall diametrically opposite the trachealis was greater than that of the muscle-containing tissue. Neither acetylcholine nor histamine significantly altered the lanthanum-resistant calcium fraction in tissue devoid of trachealis muscle.

Discussion

In many smooth muscles, tension development is associated with an increase in the frequency of discharge of spike-like potentials. Guinea-pig trachealis does not normally discharge spikes but extracellular recording (Small, 1982) revealed a close association between slow wave activity and tension development. The ability of low concentrations of acetylcholine and histamine to promote both tension development and an increase in slow wave frequency (Figures 1 and 2; Table 1) adds further weight to the argument that, in guinea-pig trachealis, slow waves may be the functional equivalent of the spikes seen in other smooth muscles.

The extracellular studies of Small (1982) showed that concentrations of acetylcholine or histamine in excess of 10 μmol l^{-1} caused a reduction and eventual abolition of spontaneous slow waves of guinea-pig trachealis. Two possible explanations were offered: acetylcholine and histamine were acting either to desynchronize slow wave discharge or to suppress slow waves by causing depolarization in individual cells. The present results clearly point to the second of these explanations as being correct and confirm Kirkpatrick's (1981) assertion that the discharge of trachealis slow waves is dependent upon resting membrane potential.

The depolarization evoked by acetylcholine and histamine is presumably a consequence of receptor activation but the role of depolarization in the subsequent production of tension is obscure. Double sucrose gap experiments using canine trachealis

Table 2 The effects of acetylcholine and histamine on the lanthanum-resistant calcium fraction of guinea-pig trachea

		Muscle-containing tissue	*Tissue devoid of trachealis muscle*
Control		0.129 ± 0.013	0.369 ± 0.032
	10	0.139 ± 0.012	0.349 ± 0.025
Acetylcholine	100	0.141 ± 0.015	0.353 ± 0.053
(μmol l^{-1})	1000	0.134 ± 0.011	0.370 ± 0.031
Control		0.135 ± 0.015	0.324 ± 0.028
	2	0.137 ± 0.015	0.375 ± 0.048
Histamine	20	0.139 ± 0.013	0.351 ± 0.020
(μmol l^{-1})	200	0.146 ± 0.016	0.385 ± 0.047

Data indicate mean tissue: medium ratio (ml g^{-1}) for $^{45}Ca^{2+}$ ± s.e. (n = 6).

(Coburn, 1979) showed that a polarizing current could offset the depolarization induced by acetylcholine yet augment the developed tension, suggesting that depolarization may not be necessary for the spasmogenic action of acetylcholine.

The electrical 'noise' evoked in many cells by high concentrations of acetylcholine or histamine was an unexpected observation. Although all cells depolarized when exposed to these agonists, 'noise' was not always observed. This suggests that the 'noise' may not be directly attributable to muscarinic or H_1-receptor activation. In view of the fast hyperpolarizing deflections apparent in the 'noise' (Figure 1) we considered the possibility that acetylcholine might activate neural nicotinic receptors and thereby evoke the release of inhibitory neurotransmitters. However, contrary to this view, acetylcholine-induced 'noise' showed no sign of fade. Furthermore, the noise was resistant to concentrations of tetrodotoxin and hexamethonium in excess of those known (Clark *et al.*, 1981) to antagonize nicotine acting on inhibitory neurones in this tissue.

Two factors suggest that the electrical 'noise' did not represent dislodgement of the microelectrode tip from the impaled cell. Firstly, dissipation of the 'noise', repolarization and recovery of slow wave discharge occurred when the agonist was washed from the tissue. Secondly, spontaneous or deliberate withdrawal of the electrode from the cell yielded an instantaneous depolarizing step followed by a stable electrical signal close to the level of zero potential difference. It is possible that the electrical noise is an artefact caused by marked tension development in the impaled cell – perhaps through contact of the microelectrode tip with intracellular surfaces. However, electrical 'noise' sometimes accompanied the actions of high concentrations of acetylcholine or histamine in the extracellular recordings of Small (1982). Clearly the mechanism underlying the electrical 'noise' will only be explained by further experimentation.

The failure of acetylcholine or histamine to increase the lanthanum-resistant calcium fraction of muscle-containing tracheal strips (Table 2) stands in marked contrast to the efficacy of potassium chloride and tetraethylammonium in identical experiments (Foster *et al.*, 1983a,b). It would seem , therefore, that during agonist-induced tension development the cellular influx of Ca^{2+} is marked in the case of potassium chloride and tetraethylammonium but undetectable in the case of acetylcholine and histamine.

This suggests that the depolarization evoked by acetylcholine and histamine does not lead to the opening of large numbers of voltage-operated Ca^{2+} channels mediating Ca^{2+} influx. Further evidence to this effect is provided by the observation (Cerrina *et al.*, 1983; Cheng & Townley, 1983; Foster *et al.*, 1984) that organic inhibitors of the voltage-operated Ca^{2+} channel (i.e. diltiazem, nicardipine, nifedipine and verapamil) can markedly depress cumulative concentration-tension curves for potassium chloride and tetraethylammonium without depressing such curves for acetylcholine and histamine. Clearly, in this tissue there is no simple link between membrane potential and the opening of the 'voltage-operated channel'.

However, the upstroke of the slow waves of guinea-pig trachealis may be Ca^{2+}-carried (Small, 1982; Foster *et al.*, 1983a; 1984). Since in the present study, both acetylcholine and histamine transiently increased slow wave frequency, we must presume that this is associated with some Ca^{2+} influx but that this is below the level of detection by the lanthanum technique.

The present results suggest that acetylcholine- or histamine-induced tension development mainly involves the release of Ca^{2+} from intracellular sites of sequestration rather than Ca^{2+} influx. However, it is possible that subsequent maintenance of that tension does require some Ca^{2+} influx, for organic calcium antagonists are able to reduce established spasm in trachealis treated with muscarinic agonists or histamine (Cerrina *et al.*, 1983; Cheng & Townley, 1983; Ahmed *et al.*, 1983; Foster & Small, unpublished observations). In view of this we propose that, for spasmogenic responses to acetylcholine or histamine, release of Ca^{2+} from intracellular sites of sequestration alone is sufficient for the development of tension but the subsequent maintenance of the developed tension may also involve Ca^{2+} influx through channels sensitive to the organic calcium antagonists.

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