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The spasmogenic action of potassium chloride in guinea-pig trachealis

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1 Tissue bath experiments showed that potassium chloride (KCl) at 10–40 mmol l^{-1} evoked spasm of guinea-pig trachealis which was unaffected by atropine (1 μmol l^{-1}), mepyramine (1 μmol l^{-1}), tetrodotoxin (3 μmol l^{-1}) or indomethacin (2.8 μmol l^{-1}).

2 Spasm evoked by KCl was depressed in Ca^{2+}-free Krebs solution or by exposure of tissues to $LaCl_3$ (0.25–1 mmol l^{-1}).

3 Extracellular electrical recording showed that the spasm evoked by KCl 10 mmol l^{-1} was associated with promotion of electrical slow wave activity. Higher concentrations of KCl abolished slow wave activity but caused further tension development.

4 Intracellular recording confirmed the ability of KCl 10 mmol l^{-1} transiently to promote slow wave activity in individual trachealis cells. This action was associated with depolarization and tension development. Higher concentrations of KCl evoked further tension development but slow waves were suppressed as the depolarization evoked by KCl increased.

5 KCl (10–40 mmol l^{-1}) increased the lanthanum-resistant calcium fraction of muscle-containing strips of trachea.

6 It is concluded that KCl acts directly on the smooth muscle of guinea-pig trachea. The spasmogenic action is associated with transient promotion of slow wave activity and a fall in resting membrane potential. The spasm involves the cellular influx of Ca^{2+} and is dependent on the presence of Ca^{2+} in the extracellular fluid.

Introduction

Smooth muscles may be classified into those that normally exhibit regenerative action potential activity and those that do not. In the latter type it has been proposed that K^+ enrichment of the medium results in graded depolarization of the cell membrane. The opening of voltage-sensitive calcium channels then causes an increase in membrane permeability to Ca^{2+}, the cellular influx of Ca^{2+} and hence the development of tension (Bolton, 1979).

Regenerative action potentials are not normally discharged by the smooth muscle of the mammalian trachea (Kirkpatrick, 1981) and there is some evidence that trachealis may respond to K^+-rich media essentially as Bolton (1979) has predicted. When challenged with KCl, canine trachealis both depolarizes in a smoothly graded way and generates tension (Suzuki, Morita & Kuriyama, 1976; Coburn & Yamaguchi, 1977). Direct evidence that KCl-promoted spasm is potential-dependent comes from experiments where current pulses which return the membrane potential towards normal also suppress the spasm (Coburn & Yamaguchi, 1977). Dependence of KCl-induced spasm on extracellular Ca^{2+} has been demonstrated in the trachealis of the ox (Kirkpatrick, 1975) and the guinea-pig (Creese & Denborough, 1981). As yet, however, there has been no direct demonstration in trachealis of K^+-promoted cellular influx of Ca^{2+}.

The present study comprises an analysis of the spasmogenic action of KCl in guinea-pig trachealis. The results of the analysis suggest that the spasm evoked by KCl represents a direct action on the smooth muscle cells. Evidence is presented that this spasm involves the cellular influx of Ca^{2+} and that most of the Ca^{2+} influx is associated with graded depolarization of the muscle cells rather than slow wave or spike activity.

Methods

Guinea-pigs (350–750 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.

Tissue bath studies

Small segments of trachea were set up for the isometric recording of tension changes as previously described (Foster, Small & Weston, 1983). The effects of spasmogenic drugs were studied by constructing cumulative concentration-effect curves. The contact time for each concentration of a particular agonist was such as to allow the development of almost all the tension rise attainable by that concentration of agonist. For acetylcholine this was 2 min, for histamine 6 min, for tetraethylammonium (TEA) 5 min and for KCl 12 min.

Lanthanum chloride ($LaCl_3$) was allowed 10 min equilibration with the tissues before agonist activity was re-examined. Other antagonist drugs or Ca^{2+}-free media were allowed to equilibrate with test tissues for at least 40 min before the effects of agonists were retested. Control tissues from the same trachea were not exposed to antagonists or Ca^{2+}-free media but otherwise were treated identically to test tissues.

Extracellular recording

Segments of trachea were set up for the extracellular recording of electrical and mechanical activity as previously described (Small, 1982). The effects of KCl 10–40 mmol/l were examined by addition of KCl to the Krebs solution superfusing the tissue. Cumulative concentration-effect curves for KCl were constructed as described for the tissue bath studies.

Intracellular recording

Simultaneous recording of intracellular electrical activity and the mechanical changes of a contiguous segment of trachea was performed as described by Dixon & Small (1983). The effects of KCl were examined by addition of KCl to the Krebs solution superfusing the tissue. Attempts to construct cumulative concentration-effect curves (as described above) were made during the microelectrode impalement of a single cell. Measurements of electrical responses were made 12 min after first exposure to each concentration of KCl. In the case of higher concentrations of KCl such measurements were often prevented by the premature dislodgement of the microelectrode from the cell under examination.

Measurement of the lanthanum-resistant calcium fraction

The method of Foster *et al.* (1983) was used to measure the lanthanum-resistant calcium fraction of muscle-containing and muscle-free strips of trachea. In the present study, however, KCl replaced TEA as the spasmogen under investigation. Hence, 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 KCl (10 mmol l^{-1}, 20 mmol l^{-1} or 40 mmol l^{-1}). Tissue strips acting as

Table 1 Failure of atropine, mepyramine, tetrodotoxin or indomethacin to antagonise KCl in guinea-pig trachealis

		LDR		
Antagonist	*Agonist*	With antagonist	Time-matched control	Net shift due to antagonist
Atropine (1 μmol l^{-1})	Acetylcholine	1.29 ± 0.22	−0.62 ± 0.16	1.91 ± 0.27
	KCl	−0.13 ± 0.04	−0.11 ± 0.03	−0.02 ± 0.05
Mepyramine (1 μmol l^{-1})	Histamine	> 1.64 ± 0.22	−0.13 ± 0.13	> 1.77 ± 0.26
	KCl	0.04 ± 0.05	−0.10 ± 0.03	0.14 ± 0.06
Tetrodotoxin (3 μmol l^{-1})	Histamine	0.04 ± 0.19	0.17 ± 0.019	−0.13 ± 0.27
	KCl	0.04 ± 0.02	−0.02 ± 0.02	0.06 ± 0.03
Indomethacin (2.8 μmol l^{-1})	Acetylcholine	−0.38 ± 0.15	−0.33 ± 0.15	−0.05 ± 0.21
	KCl	0.03 ± 0.03	0.03 ± 0.03	0 ± 0.04

Data represent $\log_{10}$ dose-ratio for the indicated agonist ± s.e. ($n = 6$); a negative sign signifies a leftward shift. > indicates that, in tissues where profound antagonism was seen, the log dose-ratio could only be observed to exceed a limiting value set by our ability to increment histamine concentration: the s.e. and mean are necessarily then imprecise.

controls were treated with vehicle; 12 min 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.*, 1983).

Drugs and solutions/statistical analysis of results

Drug concentrations are throughout expressed in terms of molar concentration of the base. Where KCl was used as a spasmogen the stated concentration excludes the KCl provided by the formulation of the physiological salt solution. The following drugs were used: acetylcholine chloride (BDH), atropine sulphate (Sigma), histamine acid phosphate (BDH), indomethacin (Sigma), lanthanum chloride (BDH), mepyramine maleate (M & B), potassium chloride (Hopkin & Williams), tetraethylammonium bromide (Sigma) and tetrodotoxin (Sigma). Stock solutions of acetylcholine and indomethacin 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+}.

Tissue bath and radioisotope studies involving the use of $LaCl_3$ were carried out in MOPS physiological salt solution (Jetley & Weston, 1980). However, most of the tissue bath and electrophysiological studies were carried out in Krebs solution as described by Small (1982). Ca^{2+}-free Krebs solution was prepared by omitting $CaCl_2$ from the formulation.

The significance of differences between means was assessed using a two-tailed unpaired *t* test.

Results

Tissue bath studies

Acetylcholine (1–1000 μmol l^{-1}), histamine (2–200 μmol l^{-1}) and KCl (10–40 mmol $^{-1}$) each evoked tonic (smoothly developing) spasm of the trachealis. The spasm evoked by KCl 10 mmol l^{-1} was, in some tissues, preceded by a small relaxation. This relaxant effect was usually seen in the initial log concentration-effect curve for KCl but not in subsequent curves in the same experiment.

While atropine (1 μmol l^{-1}) caused profound antagonism of the action of acetylcholine, it did not modify the action of KCl tested on the same tissue. Similarly, mepyramine (1 μmol l^{-1}) profoundly antagonized the action of histamine but did not affect KCl tested on the same tissue. Tetrodotoxin (3 μmol l^{-1}) was without effect on the action of KCl. Indomethacin (2.8 μmol l^{-1}) too was without effect in spite of causing complete abolition of spontaneous tone (Table 1).

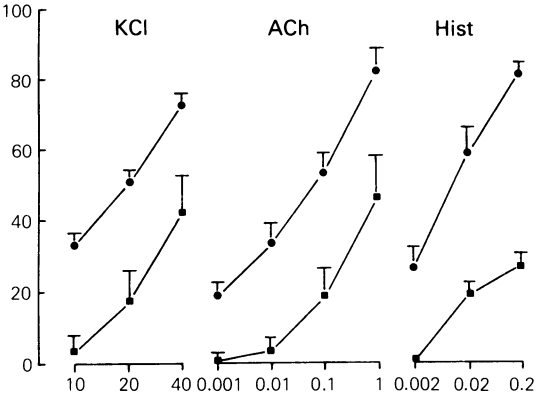

Figure 1 The effects of Ca^{2+}-free Krebs solution on the spasmogenic responses of guinea-pig trachealis to KCl, acetylcholine (ACh) and histamine (Hist). The abscissa scale represents the concentration of drug (mmol l^{-1}) on a log scale. The ordinate scale represents the response as a % of the initial maximal response to ACh. (●) = Initial log concentration-effect curve obtained in normal Krebs solution; (■) = concentration-effect curve constructed after 40 min exposure to Ca^{2+}-free Krebs solution. Data represent the means of values from 6 tissues; s.e. shown by vertical bars.

In the experiments where the effects of Ca^{2+}-free Krebs solution were examined, control experiments run at the same time showed that the shape and position of log concentration-effect curves for acetylcholine, histamine and KCl changed little when these agonists were retested following 40 min incubation in normal Krebs solution.

Exposure of test-tissues to Ca^{2+}-free Krebs solution evoked some tone loss. Following 40 min tissue incubation with Ca^{2+}-free Krebs solution, the log concentration-effect curves of acetylcholine, histamine and KCl each underwent some depression (Figure 1).

Exposure of tracheal segments to $LaCl_3$ (0.1–1 mmol l^{-1}) evoked loss of tone which proceeded more rapidly than that observed on exposure to Ca^{2+}-free Krebs solution; 10 min treatment with $LaCl_3$ evoked depression of the concentration-effect curves of acetylcholine, KCl and TEA (Figure 2). TEA was the most susceptible of the three agonists to depression by $LaCl_3$ in that 0.5 mM $LaCl_3$ affected TEA more ($P = 0.004$) than the other spasmogens. The action of $LaCl_3$ was concentration-dependent and virtually irreversible. When low

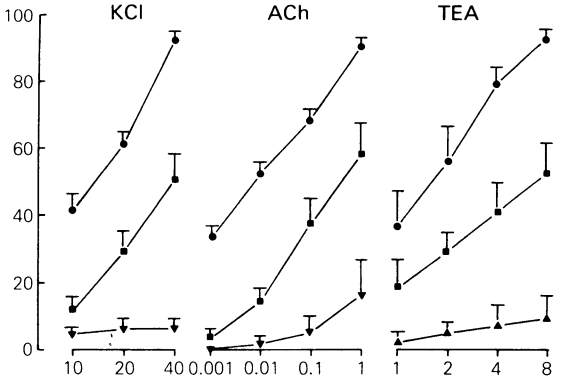

Figure 2 The effects of $LaCl_3$ on the spasmogenic response of guinea-pig trachealis to KCl, acetylcholine (ACh) and tetraethylammonium (TEA). The abscissa scale represents the concentration of drug (mmol l^{-1}) on a log scale. The ordinate scale represents the response as a % of the initial maximal response to ACh. (●) = Initial log concentration-effect curve obtained in normal Krebs solution. Other concentration-effect curves were obtained after 10 min incubation with $LaCl_3$ 0.25 mmol l^{-1} (■), 0.5 mmol l^{-1} (▲) or 1 mmol l^{-1} (▼). Data represent the means of values from 6 tissues; s.e. shown by vertical bars.

(0.1–0.25 mmol l^{-1}) concentrations of $LaCl_3$ were used it was often observed that the spasm evoked by an agonist could not subsequently be dissipated by washing the tissue with agonist- and $LaCl_3$-free medium.

Extracellular electrophysiological recording

Prior to challenge with KCl, six of the eight tissues examined did not exhibit spontaneous electrical activity. In these six tissues KCl 10 mmol l^{-1} induced both tension development and electrical slow waves. Higher (20 and 40 mmol l^{-1}) concentrations of KCl appeared to suppress the slow waves yet induced further tension development (Figure 3 and Table 2).

In the two tissues which exhibited spontaneous electrical activity, KCl 10 mmol l^{-1} evoked tension development and an increase in the amplitude of slow waves. The higher concentrations of KCl again suppressed slow wave activity while evoking greater spasm.

KCl failed to evoke electrical spike activity from any of the eight tissues examined.

Intracellular electrophysiological recording

Intracellular recording showed that some trachealis cells lacked spontaneous electrical activity while others exhibited a variety of patterns of slow wave discharge. In all tissues examined KCl 10 mmol l^{-1} evoked depolarization (15.1 ± 1.3 mV; mean ± s.e.mean, $n = 11$) of the impaled cell and spasm of the contiguous segment of trachea. The cellular depolarization was accompanied either by the initiation of slow wave discharge or by an increase in the amplitude of slow waves (Figure 4).

Higher concentrations (20 and 40 mmol l^{-1}) of KCl evoked greater depolarization of the impaled

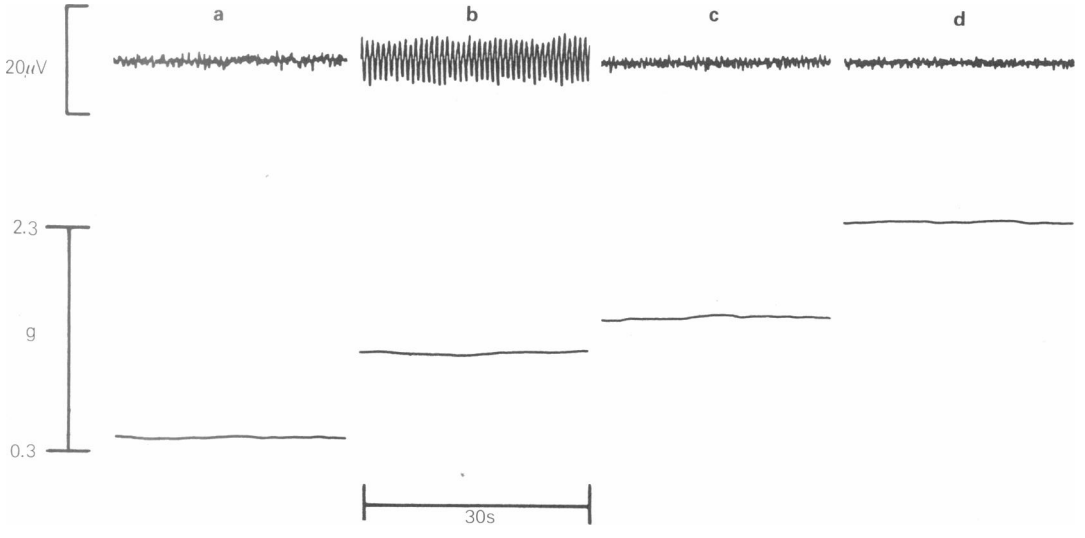

Figure 3 Guinea-pig trachealis: extracellular electrical activity (upper trace) and mechanical changes (lower trace) evoked by KCl. (a) = Control activity; (b, c, d) = activity observed 12 min after 10, 20 and 40 mmol l^{-1} KCl respectively. All records from the same preparation.

Table 2 The effects of KCl on the mechanical and extracellular electrical activity of guinea pig trachealis

	Control	KCl (mmol/l) 10	20	40
Tension developed (mg)	—	775 ± 119	1190 ± 176	1727 ± 220
Slow wave frequency (Hz)	0.33 ± 0.2	1.55 ± 0.06	0 ± 0	0 ± 0
Maximal amplitude of slow waves (μV)	1.15 ± 0.76	7.7 ± 0.87	0 ± 0	0 ± 0

Data indicate means of values from 8 tissues ± s.e. Mean values of control slow wave frequency and maximal amplitude include individual values of zero from preparations which lacked spontaneous slow wave activity before exposure to KCl.

Table 3 The effects of potassium chloride (KCl) on the lanthanum-resistant-calcium fraction of guinea-pig trachea

	Control	*KCl* (mmol/l) 10	20	40
Muscle containing tissue	0.129 ± 0.009	0.183 ± 0.014	0.216 ± 0.016	0.289 ± 0.026
Tissue devoid of trachealis muscle	0.502 ± 0.047	0.329 ± 0.070	0.235 ± 0.033	0.280 ± 0.035

Data indicate mean tissue : medium ratio (ml g^{-1}) for $^{45}Ca^{2+}$ ± s.e.mean ($n = 12$).

cell. For 20 mmol l^{-1} KCl the evoked depolarization was 33.9 ± 2.9 mV (mean ± s.e.mean, $n = 7$). In the face of marked depolarization slow wave amplitude always fell and often the discharge of slow waves ceased. Despite this, the higher concentrations of KCl evoked greater spasm from the contiguous segment of trachea.

In none of the impaled cells did KCl ever evoke electrical spike activity.

Measurement of the lanthanum-resistant calcium fraction

Table 3 presents values of the lanthanum-resistant calcium fraction of trachea. In the muscle-containing tissue strips KCl 10–40 mmol l^{-1} caused a significant ($P < 0.05$) and concentration-dependent increase in the lanthanum-resistant calcium fraction.

The lanthanum-resistant calcium fraction of tissue taken from the tracheal wall diametrically opposite

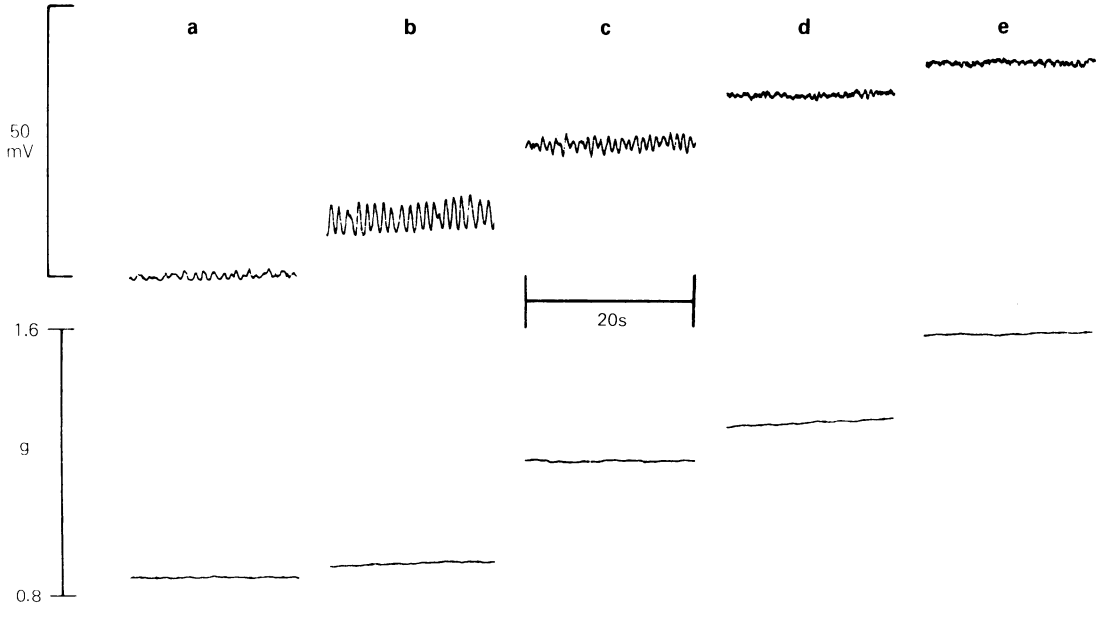

Figure 4 The effects of KCl on the intracellular electrical activity and mechanical changes of guinea-pig trachea. Upper trace = membrane potential changes. Lower trace = tension changes of a contiguous segment of trachea. (a) = Control activity prior to KCl challenge; (b, c) = activity seen 3 and 12 min respectively after KCl 10 mmol l^{-1}; (d, e) = activity seen 3 and 12 min respectively after KCl 20 mmol l^{-1}. All electrical records taken from the same cell.

the trachealis was greater than that of the muscle-containing tissue and was not increased by KCl. Indeed, the two highest concentrations of KCl evoked significant ($P < 0.05$) reduction of the lanthanum-resistant calcium fraction.

Discussion

Bolton (1979) has cited several reports of the ability of K^+-rich media to evoke neurotransmitter release. Such effects can complicate studies of the direct action of K^+ on smooth muscle. In the present experiments, the transient relaxation evoked by first exposure of some tissues to KCl 10 mmol l^{-1} was not seen on subsequent challenge with KCl. The inhibitory nature of the response and its marked tachyphylaxis lead us to suspect that it represents the release by KCl of a relaxant mediator. However, we have performed no experiments to test this hypothesis or to identify the putative mediator.

In contrast the evidence suggests that the spasmogenic effects of KCl represent a direct action on the smooth muscle cells. Tetrodotoxin (3 μmol l^{-1}) had little or no effect on the spasm evoked either by histamine or KCl. This concentration of tetrodotoxin virtually abolished the effects of field stimulation of intramural nerves in guinea-pig trachealis (Foster *et al.*, 1983). Thus, neither histamine nor KCl was causing spasm by evoking neurotransmitter release secondary to the discharge of sodium-dependent action potentials. The spasmogenic effects of KCl did not exhibit tachyphylaxis and were not significantly modified by concentrations of atropine and mepyramine causing profound antagonism of acetylcholine and histamine respectively. Nor was modification achieved by a concentration of indomethacin causing total loss of spontaneous tone. These observations strongly suggest that the excitatory action of KCl did not involve the release of mediators like acetylcholine, histamine or prostaglandins, but was a direct action on the trachealis cells.

In the trachealis of the ox (Kirkpatrick, Jenkinson & Cameron, 1975), dog (Farley & Miles, 1975) and guinea-pig (Creese & Denborough, 1981; Cerrina, Renier, Floch, Duroux & Advenier, 1982) removal of Ca^{2+} from the bathing medium markedly suppresses spasm evoked by K^+. Some workers (Creese & Denborough, 1981; Cerrina *et al.*, 1982) have reported that tracheal spasm induced by K^+ was more readily suppressed by Ca^{2+} deprivation than spasm evoked by acetylcholine. They therefore proposed that K^+-induced spasm of trachealis is primarily dependent on the cellular influx of Ca^{2+} while acetylcholine may rather act to promote the release of Ca^{2+} from intracellular sites.

The results of the present study confirm the susceptibility of K^+-induced spasm to Ca^{2+} deprivation but do not indicate that the action of acetylcholine is less susceptible to Ca^{2+} removal (Figure 1). In the latter respect our findings are in accord with those obtained by Farley & Miles (1975) in canine trachealis. Our experiments with Ca^{2+}-free Krebs solution therefore lead us to conclude that the spasmogenic action of both KCl and acetylcholine is dependent on the presence of Ca^{2+} in the bathing medium.

Kirkpatrick (1975) used $LaCl_3$ to inhibit the transmembrane passage of Ca^{2+} in bovine trachealis. Spasm evoked by acetylcholine, histamine or TEA was depressed following $LaCl_3$ treatment and the depression was greatest in the case of TEA. Our experiments suggest that in guinea-pig trachealis, too, the spasm evoked by TEA is more susceptible to $LaCl_3$ treatment than the spasm evoked by acetylcholine. However, $LaCl_3$, like Ca^{2+}-free Krebs, did not differentiate between the actions of KCl and acetylcholine.

The tissue bath experiments with $LaCl_3$ also provide some internal justification of the methodology employed in our present and previous (Foster *et al.*, 1983) measurements of the lanthanum-resistant calcium fraction of guinea-pig trachea. $LaCl_3$ 1 mmol l^{-1} virtually abolished spasm evoked by acetylcholine, KCl and TEA. It seems reasonable to assume, therefore, that the higher concentration (10 mmol l^{-1}) of $LaCl_3$ used in our measurements of the lanthanum-resistant calcium fraction (together with the conditions of low temperature) was adequate to prevent transmembrane Ca^{2+} flux and therefore to trap intracellularly any $^{45}Ca^{2+}$ which had entered cells prior to the $LaCl_3$ treatment.

Since KCl did not increase the lanthanum-resistant calcium fraction of tracheal strips devoid of muscle, the KCl-promoted increase seen in the muscle containing strips must have involved the smooth muscle rather than mucosa, connective tissue or cartilage. We therefore suggest that the KCl-induced increase in the lanthanum-resistant calcium fraction of the muscle-containing strips is a reflection of the influx of Ca^{2+} into the muscle cells during the KCl-induced spasm. This suggestion is greatly strengthened by the fact that the contact times and concentrations of KCl used in the radioisotope studies were identical to those used in the tissue bath studies, and also by the observation that KCl-induced spasm could be suppressed in a Ca^{2+}-free medium or by $LaCl_3$ treatment.

Our observation that the lanthanum-resistant calcium fraction of muscle-free tissue was higher than that of the muscle-containing tissue confirms an earlier finding (Foster *et al.*, 1983). The KCl-induced reduction in the lanthanum-resistant fraction of the muscle-free strips is not easy to explain. In view of the

high cartilage content of such strips it may represent an interaction between cartilage, Ca^{2+} and K^+. This interaction may have attenuated the muscle-associated rise in the lanthanum-resistant calcium fraction, for the trachealis-containing tissue inevitably incorporated a small amount of cartilage.

What are the electrophysiological changes which prompt or accompany the Ca^{2+} influx induced by KCl? The present experiments show that KCl shares the ability of TEA (McCaig & Souhrada, 1980; Small, 1982; Foster *et al.*, 1983) to depolarize guinea-pig trachealis and to promote slow wave activity. However, KCl did not share the ability of TEA (McCaig & Souhrada, 1980; Small, 1982; Dixon & Small, 1983) to evoke spike potentials. Since regenerative increases in membrane conductance needed for spike firing are suppressed by the high resting membrane conductance of trachealis muscle to K^+ (Kroeger & Stephens, 1975), it is not surprising that an inhibitor of K^+ conductance such as TEA can evoke spikes while the addition of KCl to the extracellular fluid cannot.

The concentration ($10\,mmol^{-1}$) of KCl causing slow wave promotion in guinea-pig trachealis corresponded with the lower part of the log concentration-tension curve. The spasm evoked by higher concentrations of KCl was accompanied solely by graded depolarization of the muscle cells. If KCl acts principally to promote the cellular influx of Ca^{2+} through potential-sensitive channels and if tension development is a reflection of the amount of Ca^{2+} entering the cell, then K^+-promoted Ca^{2+} entry must principally be associated with the graded depolarization rather than slow wave activity. A similar effect presumably occurs in canine trachealis where exposure to K^+-rich media does not evoke slow wave or action potential activity (Suzuki *et al.*, 1976; Coburn & Yamaguchi, 1977) but causes graded depolarization of up to 40 mV (Suzuki *et al.*, 1976; Coburn & Yamaguchi, 1977; Farley & Miles, 1977).

In conclusion, the spasm evoked by KCl represents a direct action on the smooth muscle of guinea-pig trachea. The spasm is associated with the influx of Ca^{2+} and it seems likely that most of the Ca^{2+} influx is linked to graded depolarization rather than slow wave activity.

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References

BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

CERRINA, J., RENIER, A., FLOCH, A., DUROUX, P. & ADVENIER, P. (1982). Effects of Ca^{2+} antagonists on guinea pig tracheal contraction induced by various agonists. In *Abstracts of the Proceedings of the International Symposium on Calcium Modulators*, Venice, p. 130.

COBURN, R.F. & YAMAGUCHI, T. (1977). Membrane potential-dependent and -independent tension in the canine tracheal muscle. *J. Pharmac. exp. Ther.*, **201**, 276–284.

CREESE, B.R. & DENBOROUGH, M.A. (1981). Sources of calcium for contraction of guinea pig isolated tracheal smooth muscle. *Clin. exp. Pharmac. Physiol.*, **8**, 175–182.

DIXON, J.S. & SMALL, R.C. (1983). Evidence of poor conduction of muscle excitation in the longitudinal axis of guinea-pig isolated trachea. *Br. J. Pharmac.*, **79**, 75–83.

FARLEY, J.M. & MILES, P.R. (1975). Effect of extracellular Ca^{2+} on the response of dog trachealis muscle to ACh and K^+. *Fedn Proc.*, **34**, 3383.

FARLEY, J.M. & MILES, P.R. (1977). Role of depolarisation in acetylcholine-induced contractions of dog trachealis muscle. *J. Pharmac. exp. Ther.*, **201**, 199–205.

FOSTER, R.W., SMALL, R.C. & WESTON, A.H. (1983). Evidence that the spasmogenic action of tetraethylammonium in guinea-pig trachealis is both direct and dependent on the cellular influx of calcium ion. *Br. J. Pharmac.*, **79**, 255–263.

JETLEY, M. & WESTON, A.H. (1980). Some effects of sodium nitroprusside, methoxyverapamil (D600) and nifedipine on rat portal vein. *Br. J. Pharmac.*, **68**, 311–319.

KIRKPATRICK, C.T. (1975). Excitation and contraction in bovine tracheal smooth muscle. *J. Physiol.*, **244**, 263–281.

KIRKPATRICK, C.T. (1981). Tracheobronchial smooth muscle. In *Smooth Muscle: an Assessment of Current Knowledge*. ed. Bülbring, E., Brading, A.F., Jones, A.W., & Tomita, T. pp. 385–395. London: Edward Arnold.

KIRKPATRICK, C.T., JENKINSON, H.A. & CAMERON, A.R. (1975). Interactions between drugs and potassium-rich solutions in producing contractions in bovine tracheal smooth muscle: studies in normal and calcium-depleted tissues. *Clin. exp. Pharmac. Physiol.*, **2**, 559–570.

KROEGER, E.A. & STEPHENS, N.L. (1975). Effect of tetraethylammonium on tonic airway smooth muscle: initiation of phasic electrical activity. *Am. J. Physiol.*, **228**, 633–636.

McCAIG, D.J. & SOUHRADA, J.F. (1980). Alteration of electrophysiological properties of airway smooth muscle from sensitised guinea pigs. *Resp. Physiol.*, **41**, 49–60.

SMALL, R.C. (1982). Electrical slow waves and tone of guinea pig isolated trachealis muscle: the effects of drugs and temperature changes. *Br. J. Pharmac.*, **77**, 45–54.

SUZUKI, H., MORITA, K. & KURIYAMA, H. (1976). Innervation and properties of the dog trachea. *Jap. J. Physiol.*, **26**, 303–320.

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