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Evidence of poor conduction of muscle excitation in the longitudinal axis of guinea-pig isolated trachea

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1 Mechanical activity was recorded from one segment of guinea-pig trachealis muscle while intracellular electrical activity was simultaneously recorded from a contiguous segment. When the tissue was stimulated by 1–32 mmol/l tetraethylammonium (TEA) it became clear that the electrical and mechanical records were not directly correlated.

2 Dual recording of mechanical activity from two contiguous segments of trachealis revealed that one tissue segment could exhibit phasic activity whilst the other could exhibit tonic activity, despite exposure to the same concentration of TEA.

3 Histological studies revealed that the trachealis muscle was organized into bundles largely separated from one another by spaces filled with connective tissue. However, muscle bundles branched and formed anastomotic connections with near neighbours.

4 It is concluded that the cell to cell spread of muscle excitation is very poor along the longitudinal axis of the trachea. The trachealis muscle as a whole does not function as a single unit. Rather it may represent a series of effector units each comprising a small number of smooth muscle bundles.

Introduction

There are two pieces of evidence which suggest that, in guinea-pig trachealis, a degree of electrical coupling exists between the component cells of a muscle bundle or of a small group of muscle bundles. Firstly, electron microscopic studies (Hoyes & Barber, 1980; Jones, Kannan & Daniel, 1980) have revealed nexi or gap junctions between neighbouring cells. Secondly, good synchrony of cellular discharge is indicated by the qualitative similarity of the electrical activity of small segments of trachealis recorded extracellularly and that of individual cells measured by intracellular microelectrodes (Small, 1982).

A method for recording simultaneously intracellular electrical activity and mechanical changes of guinea-pig trachealis (Clark & Small, 1979) relies on the insertion of the microelectrode into one segment of trachealis whilst recording mechanical changes from an adjacent, contiguous segment. When recordings of electrical and mechanical activity are made in this manner a perfect correlation between the two parameters can only be expected if there is good electrical coupling between the muscle cells in the two recording areas. Whilst using the technique for studying the effects of tetraethylammonium (TEA), we have often noticed that the recorded electrical and mechanical changes were not correlated. The present paper describes this lack of correlation and the results of some mechanical and histological studies which suggest that it results from very poor conduction of muscle excitation in the longitudinal axis of the trachea.

Methods

Electrophysiological studies

The tissue bath used in the electrophysiological study and some other aspects of the technique have been described previously (Small & Weston, 1979). The tissue was prepared for the simultaneous recording of intracellular electrical activity and mechanical changes as described by Clark & Small (1979) but it was then mounted, mucosal surface uppermost, on a perspex tissue holder (Figure 1). In the region of microelectrode impalement this holder provided tissue fixation by means of clamps placed over the cut ends of the cartilaginous rings. In this region the degree of stretch of the trachealis muscle could be adjusted by means of a tensioning screw positioned beneath the tissue.

Mechanical activity was recorded isometrically

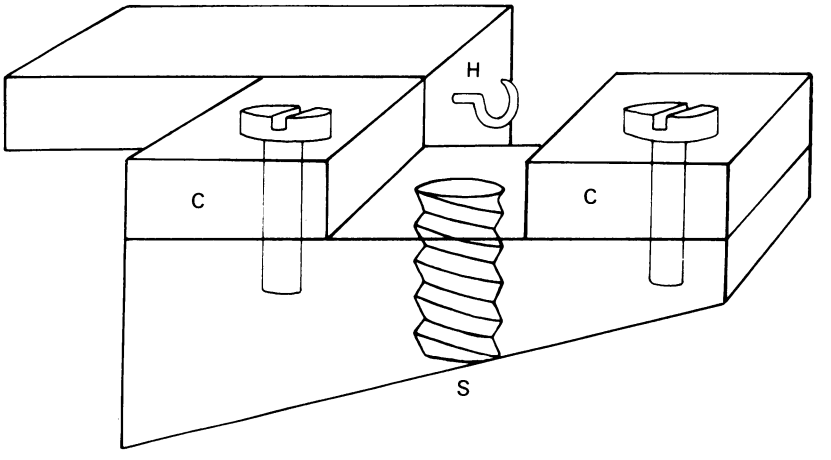

Figure 1 Perspex tissue holder used for the recording of intracellular electrical activity and mechanical changes of guinea-pig isolated trachea. Overall length of holder = 4 cm. Width of holder = 2 cm. S = tissue tensioning screw; C = clamp; H = hook for anchorage of tissue segment used for mechanical recording.

from an unclamped, contiguous segment of the tissue (Figure 2) using a 2 oz Ether transducer. The initial resting tension imposed on this tissue segment was 0.5–0.75 g.

The effects of TEA were studied in two ways. In some experiments, cumulative concentration-effect curves for TEA were constructed during the course of a microelectrode impalement of a single cell. Doubling concentration increments were used and each concentration was allowed 5 min equilibration with the tissue before a further concentration increment occurred. In other experiments a microelectrode was inserted into a cell while the tissue was bathed by TEA-containing Krebs solution. The TEA was subsequently removed from the superfusing solution, so that the effects of its washout could be studied.

Dual recording of mechanical activity

Tracheae were opened by cutting longitudinally through the cartilaginous rings diametrically opposite the trachealis muscle. A piece of trachea approximately 2.5 cm long was then prepared for the simultaneous recording of mechanical activity from a pair of contiguous tissue segments.

In the central region of the piece of trachea some cartilage and connective tissue was excised from each edge of the preparation as shown in Figure 3. Excision of cartilage in this fashion extended across several successive cartilage rings. Care was taken not to remove excessive amounts of cartilage lest the trachealis muscle was damaged in the region of its attachment near the natural tips of the cartilage rings.

The preparation was set up in the tissue bath used for the intracellular electrophysiological experiments and superfused with Krebs solution at 37.5°C. Mechanical activity was recorded isometrically from the tissue segments at the laryngeal and bronchial ends of the preparation (Figure 3) by means of a pair of 2 oz Ether transducers in conjunction with a Grass Polygraph. The tension initially imposed on each tissue segment was 0.5–0.75 g.

The spasmogenic effects of TEA 32 mmol/l were studied by adding the drug to the flow of Krebs solution. The TEA was allowed to superfuse the tissue for a period of up to 2.5 h and during this time the activity recorded from the two tissue segments was carefully scanned for signs of cross-talk. Such observation was maintained when the TEA was subsequently removed from the superfusate.

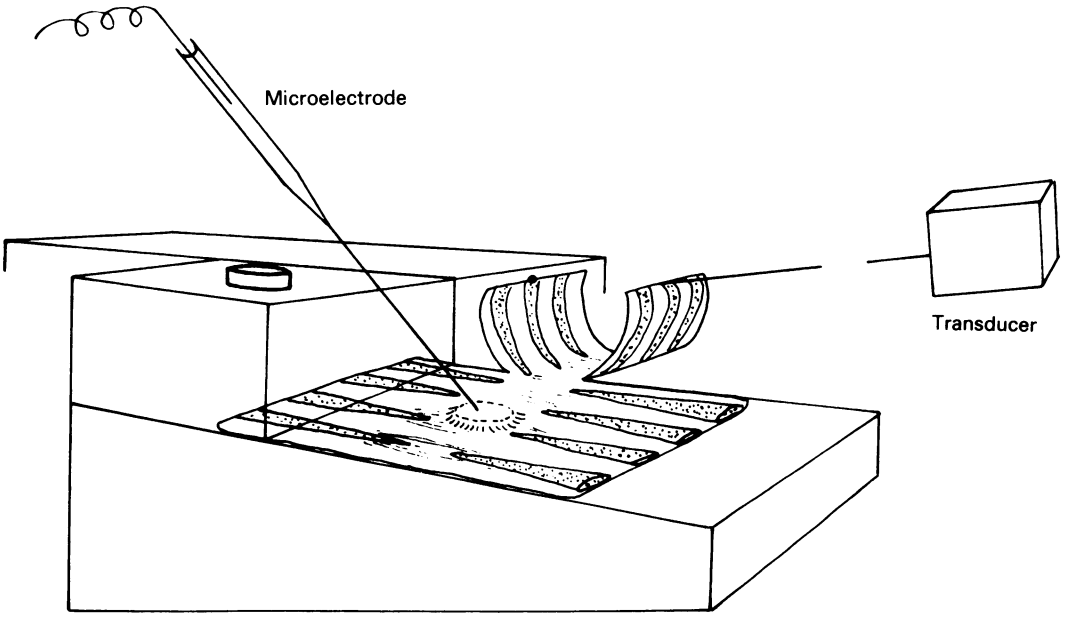

Figure 2 Arrangement of guinea-pig trachea for recording of intracellular electrical activity and mechanical changes. For the sake of clarity one of the tissue clamps has been omitted from the tissue holder. Stippled structures represent cartilage rings.

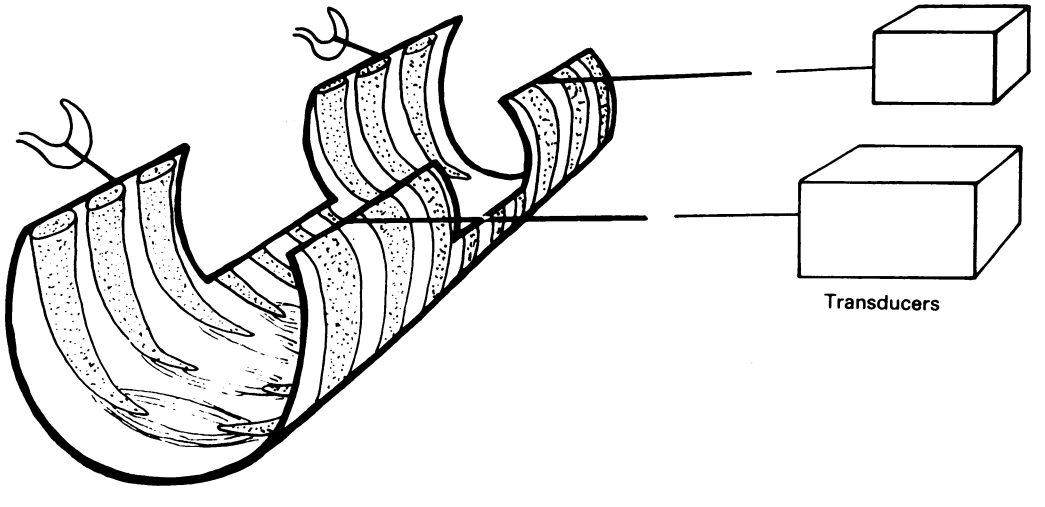

Figure 3 Technique for dual recording of mechanical activity from a pair of contiguous tissue segments of guinea-pig trachea. Stippled structures represent cartilage rings.

Histological studies

Segments of trachea, 1–2 cm long, were fixed in Bouin's fixative for 24 h before dehydration in ascending concentrations of ethanol each containing a small amount of lithium carbonate. After embedding the tissue in epoxy resin, sagittal and medial sections (2–3 μm thick) of each piece of trachea were cut, mounted on glass slides, and stained with either toluidine blue or haematoxylin and eosin.

Drugs and solutions

Tissues were superfused with Krebs solution (Small, 1982) maintained at 37.5°C and gassed with 5% CO_2 in O_2. A stock solution of tetraethylammonium bromide (TEA, Sigma) was prepared in twice distilled water.

Results

Electrophysiological studies

Concentration-effect studies for TEA were carried out during the impalement of a single cell in each of seven preparations of trachea. In all seven cells an apparent depolarization was observed on tissue exposure to TEA. This depolarization was concentration-dependent (Table 1).

One of the impaled cells did not exhibit slow wave activity prior to TEA exposure. TEA 2 mmol/l induced slow waves in this cell and higher concentrations of TEA increased the amplitude of these slow waves. Slow wave activity occurred spontaneously in the remaining six cells and in every case TEA caused a concentration-dependent increase in their amplitude (Table 1). The rising phase of the slow waves often provoked a spike potential when the concentration of TEA was 8 mmol/l or greater.

The frequency of spontaneous slow waves was relatively little changed by TEA (Table 1) but in one cell their periodic discharge was converted to a continuous discharge as the TEA concentration was increased.

The mechanical changes observed during the construction of concentration-effect curves for TEA comprised a concentration-dependent, tonic tension development in three of the preparations. In the other four preparations tonic tension development gave way to phasic mechanical activity when the TEA concentration reached 8 mmol/l.

During these concentration-effect studies it became clear that a direct correlation between the recorded electrical and mechanical changes did not exist. In the case of the tissue where the impaled cell exhibited periodic bursts of slow waves, accelerated tension development was not observed during the slow wave discharge. In tissues which progressed to phasic mechanical activity, the periods of relaxation between successive tension waves were not accompanied by changes in the pattern of slow wave discharge of the impaled cell.

A lack of correlation between recorded electrical and mechanical activity was also seen in the experiments where the effects of high concentrations of TEA were studied together with the effects of drug removal from the superfusate. Tonic discharge of action potentials could be recorded from an impaled cell throughout a period when the contiguous tissue segment was exhibiting phasic mechanical activity (Figure 4). Sometimes an impaled cell exhibited phasic electrical activity. The phasic nature of the electrical record was manifest as low amplitude slow wave activity interrupted by periods where the slow wave amplitude suddenly increased greatly. The

Table 1 The effects of tetraethylammonium (TEA) on the electrical activity of guinea-pig trachealis.

	Control	*TEA* (mmol/l)			
		1	2	4	8
Maximum amplitude of slow waves (mV)	2.5 ± 1.3	8.5 ± 2.5	15.7 ± 2.5	21.5 ± 2.1	22.3 ± 2.3
Slow wave frequency (Hz)	0.87 ± 0.15	1.02 ± 0.07	1.09 ± 0.05	1.17 ± 0.04	1.12 ± 0.08
Apparent depolarization (mV)	—	7.0 ± 1.6	10.5 ± 2.5	17.1 ± 2.7	25.0 ± 3.2

The data represent the mean values from at least 5 cells ± s.e. Each measurement was made 5 min after exposure of the tissue to the indicated concentrations of TEA.

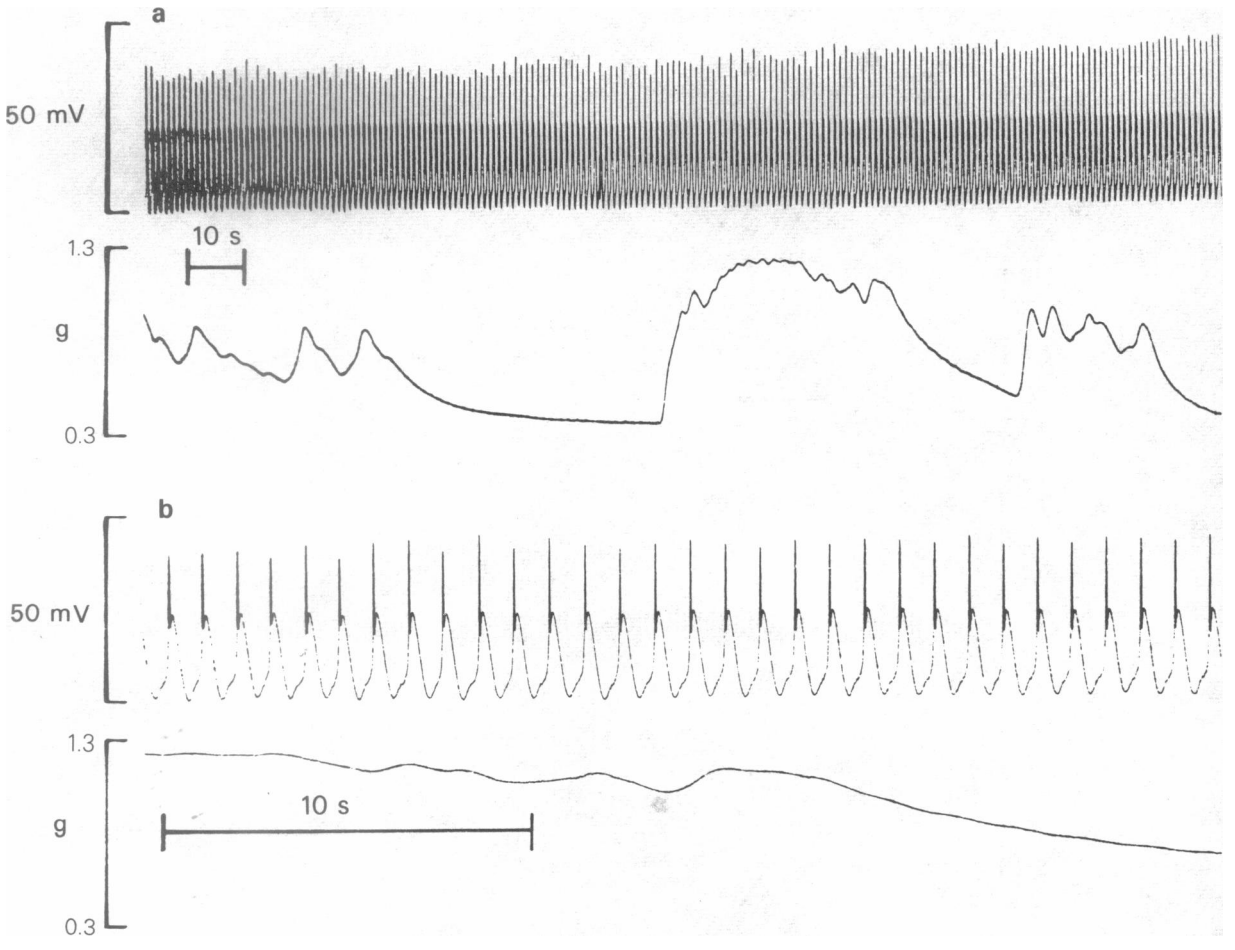

Figure 4 Recording of intracellular electrical activity and mechanical changes of guinea-pig trachea exposed to tetraethylammonium (TEA) 16 mmol/l. Upper trace = membrane potential changes; lower trace = tension changes; (b) represents a section of (a) run at a higher chart speed. Note tonic discharge of action potentials but phasic mechanical activity.

early members of the groups of large slow waves often gave rise to a spike potential. Electrical activity of this kind could be accompanied by phasic mechanical activity, but generally the appearance of high amplitude electrical activity was not synchronized with the phasic tension waves (Figure 5).

Dual recording of mechanical activity

Tracheae from 10 animals were set up for simultaneous recording of mechanical activity from a pair of contiguous tissue segments. TEA 32 mmol/l evoked contraction of both the laryngeal and bronchial tissue segments in all ten preparations.

In one preparation the TEA-induced contractions of the laryngeal and bronchial segments started simultaneously (Figure 6). In six preparations contraction of the bronchial segment preceded that of the laryngeal segment by more than 30 s. In one instance the times of onset of contraction differed by 3.5 min. In the remaining three preparations contraction of the laryngeal segment preceded that of the bronchial segment by more than 30 s.

Initially the contractions of the laryngeal and bronchial tissue segments evoked by TEA were tonic in nature. However, in two preparations (e.g. Figure 6) the laryngeal segment subsequently began to exhibit phasic mechanical activity in the presence of TEA. In

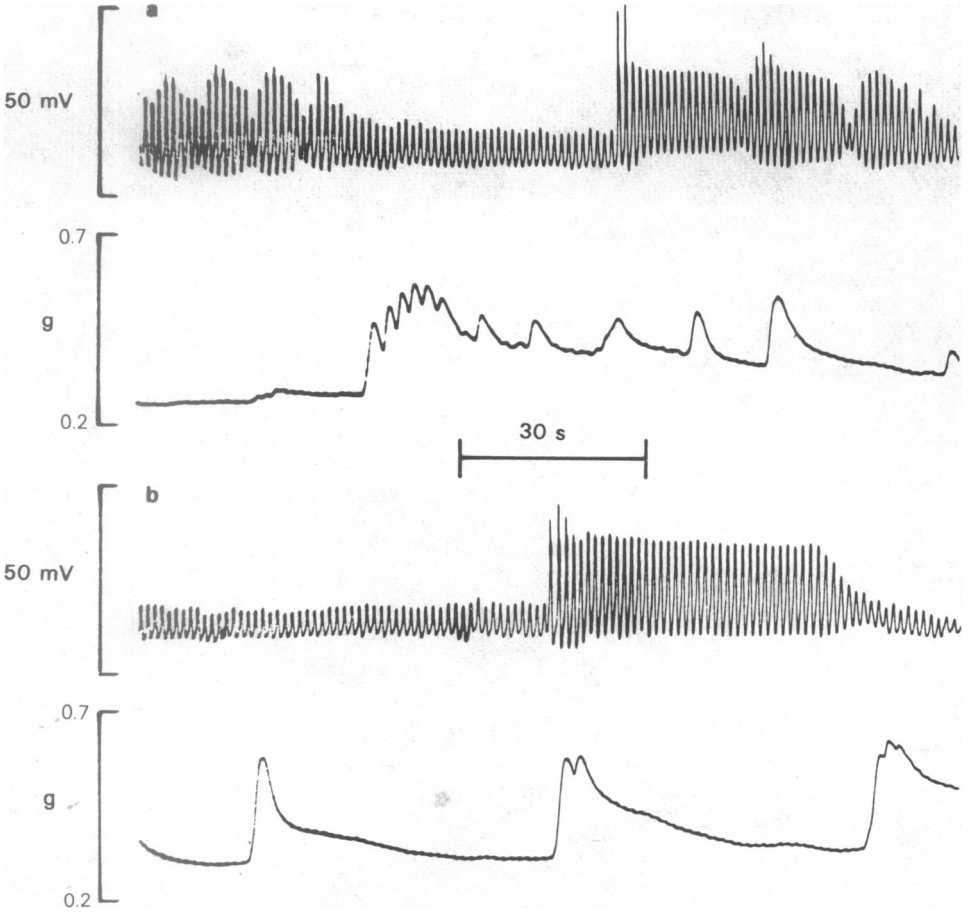

Figure 5 Recording of intracellular electrical activity and mechanical changes of guinea-pig trachea. The preparation had been exposed to tetraethylammonium 8 mmol/l but the recording illustrated is from a period during which the drug was being washed from the tissue. Upper trace = membrane potential changes; lower trace = tension changes. Note phasic activity apparent in both the electrical and mechanical records but that the two records are not correlated. Panel (b) is a continuation of the record in (a).

one preparation both the laryngeal and bronchial segments subsequently developed phasic activity in the presence of TEA.

On washout of TEA, phasic mechanical activity was exhibited by both the laryngeal and bronchial segments of five preparations. In four preparations phasic activity was recorded only from the laryngeal segment. In one preparation phasic activity was recorded only from the bronchial segment. Providing that an adequate period of washing in TEA-free Krebs solution was allowed, all tissue segments eventually lost phasic activity and assumed a level of tone close to the pre-TEA value (Figure 6).

In none of the ten preparations did the mechanical activity of the laryngeal and bronchial segments share a common time course or pattern of contraction or relaxation. This held true both for the period of TEA exposure and for the period during which TEA was washed from the preparation.

Histological studies

The guinea-pig trachealis muscle was organized into discrete bundles. The majority of the muscle bundles were circularly orientated with respect to the lumen of the trachea and extended between the tips of the

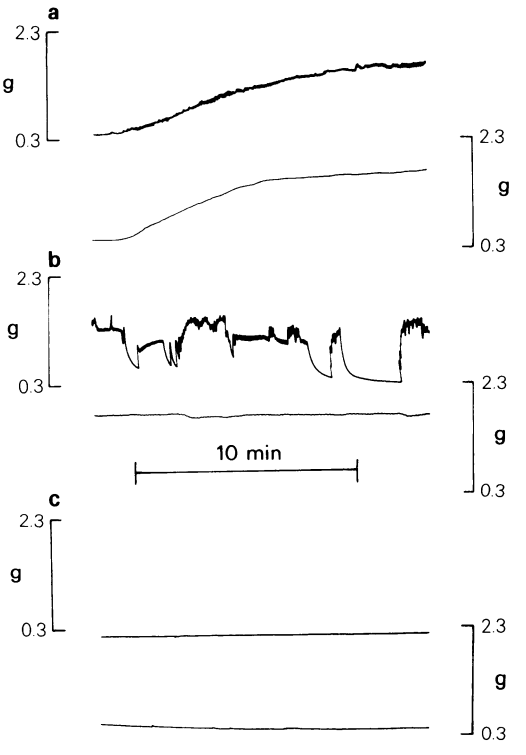

Figure 6 Dual recording of mechanical activity from a pair of contiguous tissue segments of guinea-pig trachea. Upper record = laryngeal segment; lower record = bronchial segment. (a) Initial exposure of tissue to tetraethylammonium 32 mmol/l; (b) after 2 h exposure of tissue to TEA 32 mmol/l; (c) 15 min after removal of TEA from superfusate. Note (in panel b) phasic activity adopted by laryngeal tissue segment while bronchial segment retains tonic activity.

cartilaginous rings. Many of the muscle bundles were anchored to the perichondrium near the tips of the cartilaginous rings, though some appeared to end in the loose connective tissue between adjacent rings.

Saggital sections of the trachea revealed that the smooth muscle bundles were separated from one another by spaces filled with connective tissue (Figure 7a). However, in medial sections cut in the plane of the trachealis (Figure 7b) it could be seen that many bundles were branched. A single muscle bundle could thus be anchored to the tips of two adjacent cartilaginous rings. Muscle bundle branches also formed anastomotic connections between neighbouring bundles. The trachealis muscle bundles were thus organized into a loose mesh-work.

Discussion

There is currently little or no evidence to suggest that TEA acts on tracheal smooth muscle to uncouple membrane potential changes from tension development. Kroeger & Stephens (1975) observed that tension waves could precede intracellularly-recorded action potentials in TEA-treated canine trachealis, but these workers were able to explain the observation in terms of slow conduction of the action potential through the tissue. Our own extracellular recording of the electrical and mechanical activity from very small (one or two cartilaginous rings) segments of guinea-pig trachealis (Small, 1982) did not indicate electro-mechanical uncoupling by TEA and lends support to Kirkpatrick's (1981) statement that TEA-induced potential oscillations are correlated with mechanical changes in tracheal muscle.

In view of these observations, the present lack of correlation between intracellularly-recorded electrical activity and the mechanical activity of a contiguous segment of trachea can only be explained by the absence of a conductive pathway between the muscle cells in the two recording areas. The results of our present mechanical and histological studies tend to confirm that this is so.

When mechanical activity was recorded simultaneously from two contiguous tissue segments, no cross-talk between the segments was detected in any of the ten preparations. It might be argued that the removal of cartilage between the two recording areas disrupted coupling between adjacent muscle bundles. However, this argument is negated by the histological observation that the muscle bundles terminate near the tips of the cartilage rings, and great care was taken not to remove cartilage in this region.

The absence of muscle cross-talk in the mechanical studies is supported by the histological observation that the trachealis muscle is organized into discrete bundles separated by relatively wide spaces filled with connective tissue. Furthermore the longitudinal axis of the muscle bundles is at right angles to the longitudinal axis of the trachea. These findings confirm the earlier histological study of Hoyes & Barber (1980), and would argue very effectively against the propagation of muscle excitation along the longitudinal axis of the trachea. However, this is offset to a certain extent by our finding that there are anastomotic connections between adjacent muscle bundles and that some muscle bundles link the tips of two adjacent cartilage rings, rather than the tips of a single ring. The muscle bundles can therefore be regarded as being organized into a loose meshwork.

The results of our experiments indicate very poor spread of muscle excitation through this meshwork in the longitudinal axis of the trachea. While some cell to cell spread of muscle activity undoubtedly occurs

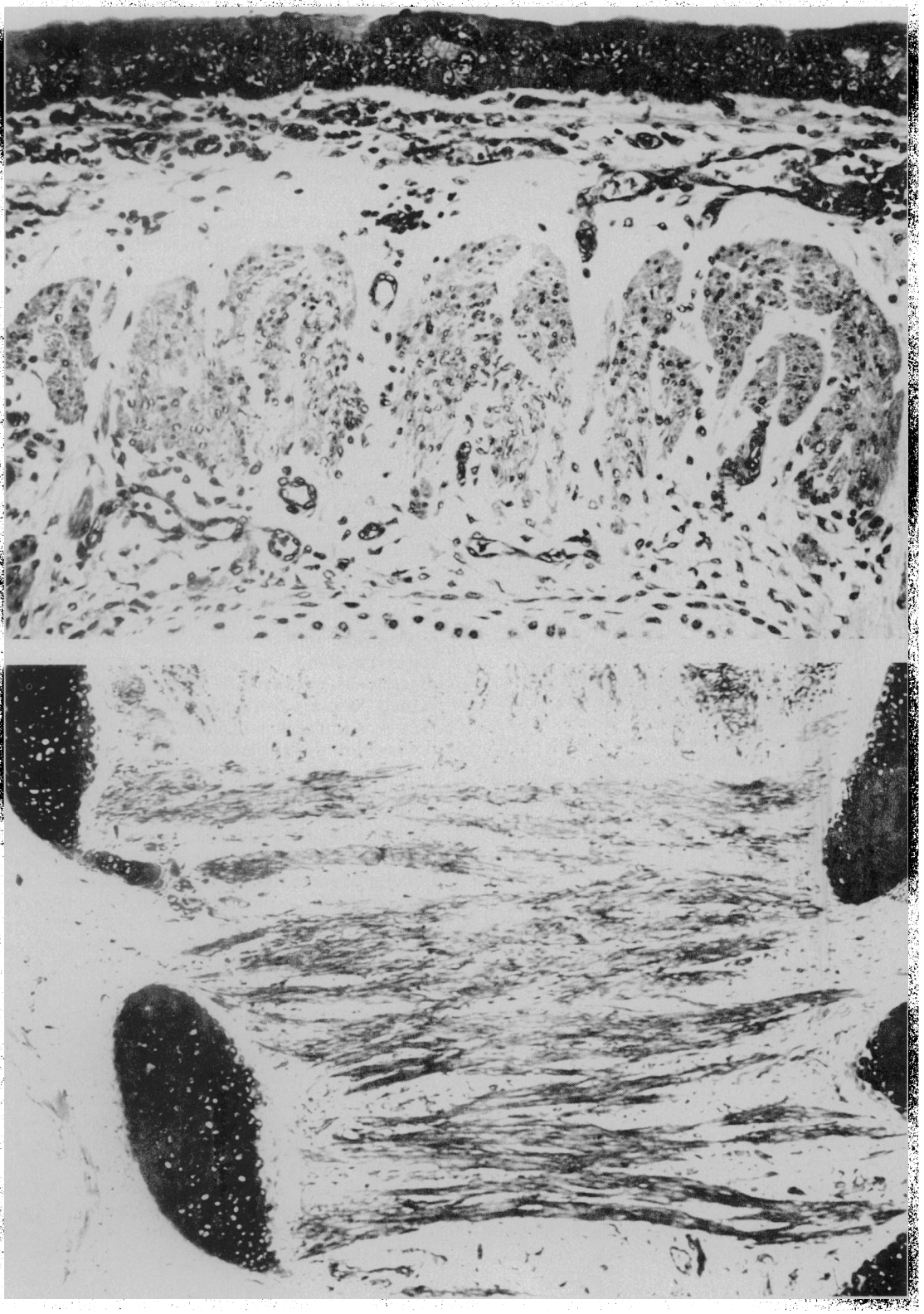

Figure 7 (a) Saggital section through guinea-pig trachea. Stain = haematoxylin and eosin. Magnification approximately 230 ×. Darkly staining material at upper edge of field = mucosa. Cartilage visible at lower edge of field. Trachealis muscle bundles cut in transverse section can be seen approximately in centre of field. (b) Medial section through guinea-pig trachea in the plane of the trachealis muscle. Stain = toluidine blue. Magnification approximately 230 ×. Darkly staining structures to left and right of field = cartilage cut in transverse section. Smooth muscle bundles which branch and anastomose can be seen running between the segments of cartilage.

in guinea-pig trachealis, the trachealis muscle as a whole does not behave as a single unit. Rather it may be organized into a series of poorly-connected or disconnected effector units, each comprising a small group of muscle bundles. Such an arrangement would certainly explain the ability of contiguous tissue segments to behave so differently, despite exposure to the same concentration of TEA. Clearly it also represents a limitation inherent to our technique for simultaneously recording intracellular electrical and mechanical activity. A good correlation between these two parameters can only be expected when it becomes possible to insert the microelectrode into the muscle bundles from which the mechanical record is obtained.

The demonstration of a correlation or lack of correlation between intracellular electrical and mechanical activity is very difficult in trachealis muscle which has not been exposed to TEA. Spontaneous tension changes are very slow and tonic in nature. Some of the impaled muscle cells are electrically quiescent whilst others exhibit slow wave activity of low amplitude.

It has been the ability of TEA to evoke phasic activity from one segment of trachea but tonic activity in a contiguous segment that has most prompted us to propose the existence of multiple effector units within the tissue. Guinea-pig trachealis does not exhibit phasic activity spontaneously or if driven by agonists such as acetylcholine, histamine or potassium chloride. Clearly then, detection of the activities of different effector units in a piece of trachea is optimized by exposure to TEA.

We remain puzzled as to why TEA sometimes evokes phasic rather than tonic mechanical activity. The electrical events associated with phasic mechanical activity may well be of the type illustrated in Figure 5. If this is so, it is likely that this kind of electrical activity will be seen in most of the cells of that effector unit since the amplitude of the phasic tension waves often approaches the maximal tension attainable by that tissue segment.

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