Induction of a myogenic response in tonic airway smooth muscle by tetraethylammonium chloride

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The realization that the tracheobronchial airways are not merely passive conduits but that by contraction of their smooth muscles they control the distribution of ventilation to the alveoli has raised many questions as to the precise nature of this control (27, 32, 33). Since the airways may be subjected to considerable and sudden transmural forces, we considered it of interest to investigate the effects of sudden changes in length on the mechanics of airway smooth muscle: specifically, we were interested to know their smooth muscles they control the distribution of ventilation to the alveoli has raised many questions as to the formation of airway caliber), it must be admitted that since

The aims of the present investigations were to determine 1) whether a MR could be elicited in TSM in vitro normally or under experimental conditions, 2) the effect of a membrane stabilizer such as Mg2+ on the production of the MR, and 3) the Ca compartment mobilized in producing the MR. The latter was of special interest as multiunit arterial smooth muscles usually contain a large amount of sequestered Ca in comparison to single-unit preparations (10).

The results suggest that 1) a MR cannot be elicited normally in TSM, but that in the presence of TEA phasic electrical and mechanical activity is produced and a MR can be easily elicited; 2) the MR cannot be elicited in solutions in which the membrane permeability is increased; 3) the Ca mobilization that is associated with depolarization is responsible for the MR.

METHODS

Tracheal smooth muscle was obtained from the cervical tracheae of mongrel dogs anesthetized with 30 mg/kg pentobarbital administered intravenously. Parallel-fibered muscle strips about 1 cm x .1 cm x .075 cm were dissected out, mounted, and equilibrated in a Krebs-Ringer bicarbonate solution of the following composition, in millimoles per liter: NaCl, 115; NaHCO3, 23.8; KH2PO4, 1.2; KCl, 4.7; MgCl2, 9.5; CaCl2, 2.5; dextrose, 5.5. The solution was equilibrated with a 95% O2-5% CO2 gas mixture so as to maintain a measured bath Po2 of 600 mmHg.
**Myogenic Response in Multiunit Smooth Muscle**

$P_{CO_2}$ of 40 mmHg, and pH 7.40 at a temperature of 37 ± 1°C. In preparing solutions containing TEA, TEA was substituted for NaCl on an equimolar basis. Low-Ca and low-Mg solutions were prepared by reducing, to the extent indicated, the amount of these respective salts added to the bathing solution.

Quick stretches for eliciting the myogenic response were applied with a Levin-Wyman ergometer. The vertical muscle strip was fixed rigidly to a clamp below. The upper end was attached by a short segment of jeweller's chain to a force transducer (RCA 5734) which was mounted firmly on the lever of the ergometer. The compliance of the system, including the force transducer and attachments to the muscle, was 2 μm/g wt. The amount of stretch applied was measured with a linear displacement transducer (Hewlett-Packard model 7DCDT-500), the displacement-measuring core of which rested on the lever of the ergometer. With this ergometer, quick stretches of given magnitudes and velocities could be applied. The signals from the RCA 5734 valve and linear displacement transducers were recorded in parallel on a Brush Mark 280 oscillograph and Tektronix model 564 oscilloscope.

**RESULTS**

**Experiments Using Normal Krebs-Ringer Bicarbonate Solution**

Guinea pig taenia coli (single-unit smooth muscle). In order to ascertain that our equipment could measure a MR in a smooth muscle preparation which is known to demonstrate such a response, our initial experiments investigated the effect of quick stretch on the taenia coli of the guinea pig. Figure 1A shows an instantaneous rise and decay of tension at the time of application of the stretch followed after a short latency by an active contraction or myogenic response (MR). The time course of the MR is influenced by the initial length of the muscle as shown. In both instances the velocity of stretching was more than 5 times the maximum shortening velocity of smooth muscle (33).

These results indicate that the equipment employed can elicit and measure an MR in a unitary smooth muscle such as taenia coli. Subsequent experiments were carried out on tracheal smooth muscle.

**Canine Tracheal Smooth Muscle (Multiunit)**

Effect of initial muscle length, rate, and magnitude of stretch. The results of these studies (Fig. 1, B–D) showed that, although the velocity of quick stretching was up to 20 times that of the maximum shortening velocity of the muscle (33), a MR could not be elicited from this muscle within muscle lengths to 1.24 L0 and magnitudes of stretch to 0.25 L0 in normal solution. It should be noted that the series-elastic component of trachealis is 7.5% of L0 (34), considerably less than the magnitude of stretch imposed, and thus the quick stretch was not merely absorbed by the series elasticity.

Effect of experimental conditions and agents which help elicit a myogenic response. Hypoxia. Loofbourrow et al. (23) have demonstrated spontaneous rhythmic tracheal contractions induced by hypoxia in the intact dog. This observation coupled with our finding that severe hypoxia may cause a small increase in resting tension (22) and the finding of others that a myogenic response is elicited more easily in a partially contracted muscle led us to investigate the effect of hypoxia on the response of tracheal smooth muscle to stretch. Using appropriate gas mixtures, the Krebs-Ringer solution was equilibrated in a reservoir and then perfused into the bath. The $Po_2$ was 30 mmHg, $Pco_2$ 40 mmHg, and pH 7.40. We have previously shown (31) that a $Po_2$ of 60 mmHg impairs tension development by 40%. However, the hypoxia used here did not elicit the myogenic response, even though contractile function was impaired.

Acidosis. In the presence of a bathing solution at pH 6.6–6.8, active responses to stimulation were impaired, but a myogenic response could not be elicited.

Potassium. Treatment with 59 mM K (59 mM NaCl replaced with KCl), which resulted in a membrane depolarization from −51 to −23 mV (unpublished obser-

**Fig. 1.** Effect of quick stretch on guinea pig taenia coli (panel A) and canine trachealis (panels B–D). In panel A responses to stretches of 0.1 and 0.25 L0 are shown. In panels B–D are shown effects of increasing initial muscle length from 0.75 to 1.25 L0 (panel B), increasing velocity of stretch from 0.5 to 5 times $V_{max}$ (panel C), and increasing magnitude of stretch from 0.1 to 0.25 L0 (panel D).

**Fig. 2.** Effect of TEA (33 mM) on mechanical activity of TSM and response to stretch. Upper record: stretch applied by a Levin-Wyman ergometer. Lower record: muscle tension as a function of time. Responses to TEA were recorded after 5 min exposure to this agent.
acetylcholine. When treated with acetylcholine (10⁻⁸ to 10⁻⁴ M), the muscles responded with dose-dependent tonic contractions, but a myogenic response could not be elicited under these conditions.

TETRAETHYLAMMONIUM CHLORIDE (TEA). Shortly after the application of TEA (33 mM), TSM exhibited rhythmic bursts of partially fused twitches, and the basal tension between contractions was usually increased (Fig. 2). The frequency of these spontaneous contractions and the increase of basal tension were widely variable between preparations. A myogenic response to quick stretching was easily elicited. The MR was initiated 1.5–3 s after the application of the stretch, and its duration was typically 3–4 s and magnitude was about 10% of P₀. Atropine, 10⁻⁵ M, did not affect the mechanical activity of MR observed in the presence of TEA.

Effect of (Mg⁺²) on response to TEA. Since the phasic mechanical responses to TEA suggested the involvement of an action potential (21), we decided to investigate the effects of Mg⁺², a membrane stabilizer (6) on the responses to TEA.

In measuring the dose-response relation to TEA in preliminary experiments, we noted that at subthreshold concentrations of TEA which did not produce an increase in tension exposure to a short electrical stimulation resulted in phasic mechanical activity following this stimulation. Since this effect is never seen in untreated TSM, this observation indicated an effect of TEA. The dose-response relationship to TEA was thus determined at normal (2.5 mM) and reduced (0.5 mM) Mg⁺² by stimulating the muscle electrically (60 Hz) for 2 s and then measuring the area under the myogram which followed (by planimetry). The response observed at a concentration of 67 mM TEA was arbitrarily assigned the value 100%. Although higher concentrations of TEA yield slightly greater responses, these are difficult to interpret because of the deficiency of these solutions in Na. A 10-min rest was allowed between successive doses of TEA and no evidence of desensitization was seen. The results (Fig. 3) show a threshold for the effect of TEA at 8 mM. Of special interest was our finding that the sensitivity of the muscle to TEA was increased (about fourfold) by reducing the (Mg⁺²)₀ to 0.5 mM. This effect was readily reversible. Decreasing (Mg⁺²)₀ below 0.5 mM had little additional effect on the dose-response relationship.

Effects of Ca, Mg, and D-600 on MR. These studies were undertaken to elucidate 1) the role of the physiological divalent cations on the effect of TEA, and 2) the Ca pool utilized in the production of the MR. In order to interpret the effects of Ca and Mg on responses to TEA, it was necessary to consider their interrelated effects on membrane permeability, the role of Ca in excitation-contraction coupling, and the apparent inhibition by Mg⁺² of responses to TEA (documented above). We thus decided to investigate the effect of reducing (Mg⁺²)₀ from 2.5 to 0.5 mM on the (Ca⁺²)₀ required for the MR. The results shown in Fig. 4 indicate that 1) the MR is quantitatively related to (Ca⁺²)₀ and has an absolute requirement for (Ca⁺²)₀, 2) the Ca threshold for the MR is inversely dependent on (Mg⁺²)₀, and 3) Ca in concentrations above 0.5 mM can substitute for Mg in supporting the membrane permeability requirements for the MR.

Since the MR demonstrated a quantitative relation to (Ca⁺²)₀ in these studies, we considered it of interest to define in greater detail the mechanism of Ca mobilization which results in the MR. An increased intracellular free Ca might result from 1) a nonspecific increase in membrane permeability, 2) a specific depolarization-activated Ca influx, or 3) a mobilization of sequestered Ca by depolarization (17, 30). In order to rule out the first possibility, we used the substance D-600 which has been shown to inhibit selectively the depolarization-activated Ca influx without affecting the resting permeability of the membrane in both cardiac muscle (19) and uterine smooth muscle (20). The results (Fig. 5) show that D-600 inhibits the MR completely.

Discussion

Effect of Quick Stretch on TSM in Control and Test Conditions

Any experimental condition that allows a transient increase in the ionic permeability of the membrane nonspecifically in response to stretch should, because of the strong inwardly directed electrochemical gradient for Ca,
evoke a MR. The refractoriness of TSM to quick stretch even in the presence of various direct agonists is somewhat surprising. Thus it is of interest that TEA, which produces a relatively small change in basal tension, can produce such a fundamental change in its biophysical properties, producing spontaneity and a myogenic response to stretch. The mechanism whereby TEA effects this change probably involves two levels of the control of muscle function: 1) its effect on membrane potassium conductance (21), and 2) the ionic requirements of excitation-contraction coupling. The present study focusses on the latter.

**Interrelation of Ca and Mg in the MR**

The complex interrelation of Ca and Mg in the effects of TEA and the MR are consistent with dual effects of both Ca and Mg on membrane permeability, sensitivity of the muscle to TEA, and excitation-contraction coupling. Thus Mg inhibits the effect of TEA as is shown in Fig. 3 but also helps to stabilize the permeability of the membrane in solutions deficient in Ca as has been shown by Bulbring and Tomita (6) in the taenia coli. A normal membrane permeability is apparently a prerequisite for the processes supporting the MR; an action potential with a low safety factor would be a likely candidate for this process (21). Calcium, which also stabilizes membrane permeability, couples excitation and contraction, and thus the observation that the MR is dependent on \((\text{Ca}^{2+})_n\), within concentrations at which the electrochemical gradient for Ca is inward provides additional evidence that the MR is dependent on membrane phenomena. This is of special interest in that multiunit smooth muscle usually contains a relatively large store of sequestered Ca which is resistant to depletion and can be mobilized by drugs for contraction (10, 22). The reasons why it is not mobilized by a quick stretch are not clear, and one might speculate that 1) there are no direct electrical connections between the membrane and these Ca stores, assuming that quick stretch does produce depolarization (7), or 2) that the mobilization of sequestered Ca by depolarization requires the release of “trigger Ca” which is subject to depletion in Ca-deficient solutions (17). Hinke (16) has also proposed that depolarization results in contraction by increasing Ca influx, whereas sequestered Ca is mobilized primarily by stimulatory agonists.

Additional experiments were then designed to elucidate the mechanism whereby the stretch produced an increase in intracellular free Ca. Thus the Ca mobilized might result from 1) a nonspecific increase in membrane permeability which results in depolarization and Ca influx in parallel, 2) a specific activation of Ca channels by depolarization which provides activator Ca for contraction, or 3) a release of trigger Ca which mobilizes sequestered Ca (17). The substance D-600, which has been shown to inhibit the activation of Ca influx by depolarization in other preparations (19, 20), promised to be a useful tool in differentiating among these possibilities if it could be shown that D-600 is selective in inhibiting only the depolarization-activated Ca influx. In testing this assumption our preliminary experiments on both trachealis and myometrium have shown 1) that D-600 inhibits potassium-induced contractions to a greater extent than it inhibits acetylcholine-induced contraction, and 2) that D-600 does not inhibit Ca sensitive actomyosin ATPase activity (R. Bose, personal communication) or contractions induced by the Ca ionophore A23187 (cf. 26). In other preparations, it has been shown that D-600 minimally affects Ca binding by isolated cardiac sarcoplasmic reticulum at 100 times the pharmacologically effective dose (11, 36) and that D-600 inhibits only the entry of the trigger Ca associated with action potentials in the taenia coli (24). Thus the evidence available indicates that the inhibitory effect of D-600 is exerted primarily through an inhibition of depolarization activated Ca influx rather than through effects on intracellular structures concerned with Ca metabolism and contraction (36). The observation that D-600 completely inhibited the MR thus argued against the possibility that a nonspecific increase in membrane permeability might explain the MR. However, since the relationship between the relative roles of trigger Ca and the Ca current of depolarization in initiating contraction, as well as the effect of D-600 on the release of trigger Ca are not yet established, these experiments could not differentiate between the latter two possibilities.

In conclusion, one might speculate that the potential for spontaneity as unmasked by TEA in the normally multiunit TSM might have some physiological significance such as the autoregulation of air flow in the smaller airways or possibly the stabilization of airways in conditions associated with the sudden development of large transmural pressures.

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