MECHANISM OF POTENTIATION OF MECHANICAL RESPONSES BY TETRAETHYLAMMONIUM IN CANINE TRACHEAL SMOOTH MUSCLE

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Abstract—The mechanism of potentiation of mechanical activity by tetraethylammonium chloride (TEA) was investigated in canine tracheal smooth muscle. Twitch tension induced by direct electrical stimulation was potentiated by 1 mM TEA to more than twice the amplitude. Although the tracheal strips usually exhibited no myogenic response to quick stretch, application of TEA (≥10 mM) produced myogenic responses to quick stretch. This response, however, could be induced in the presence of less than 3 mM TEA in a solution containing 10.9 or 15.9 mM potassium. TEA (2 mM) significantly potentiated the contraction induced by 20 mM potassium, and the potentiation was not observed in the absence of calcium ion. On the other hand, 2 mM TEA did not potentiate the contraction induced by CaCl₂ in strongly depolarized muscle. Tetramethyl- (TMA) and tetrapropylammonium (TPA) did not produce myogenic responses to quick stretch, and there was no potentiating effect on the responses to direct electrical stimulation or to high levels of potassium. The blocking activity of these two ammonium ions on ⁸⁶Rb-efflux was considerably less than seen with TEA. In conclusion, the blocking of potassium conductance by TEA, even at concentrations lower than 5 mM, results in the increase in Ca conductance when electrically, mechanically or chemically stimulated. TEA probably increased the transmembrane Ca-influx as a result of the potentiation of cell membrane depolarization.

Tetraethylammonium (TEA) has a profound blocking effect on potassium conductance (gK) in various excitable tissues (1–3). The effects of TEA on smooth muscles have also been investigated from various aspects, and studies have been extensively carried out in smooth muscles having a cell membrane with a low excitability (2). Canine tracheal smooth muscle belongs to such a group of smooth muscles, and the strong rectifying property of the cell membrane to the depolarizing current may be responsible for its stable resting membrane potential and for the lack of induction of action potentials (4, 5). In the presence of TEA, however, excitation readily occurs, either in response to quick stretch, by electrical stimulation, or spontaneously (6–9). TEA induces Ca spikes when the membrane is depolarized to the threshold of the action potentials by reducing the potassium conductance and unmasking the Ca inward current (4). Most of these effects of TEA have been observed only under conditions with high concentrations of the drug (10–40 mM).

On the other hand, Kalsner (10) found that contractions induced in the rabbit aorta by various agonists were notably potentiated by TEA at very low concentrations (0.8 mM), and he suggested that TEA directly interacts with Ca mobilization as a quaternary ammonium ion and increases Ca utilization by
the smooth muscle. Similar notable sensitization to various agonists by low concentrations of TEA has been found in aortic strips from both normal and reserpinized rabbits (11). In a previous paper, we showed that TEA interacts with the site of action which is responsible for K permeability (may be the K channel itself) in the depolarized cell membrane of canine tracheal smooth muscle, and we found that the dissociation constant for TEA binding (1 mM) was far less than the concentration at which spontaneous electrical activities were produced (10–30 mM, (12)). Of interest was the unclear relationship between the two effects of TEA: the blocking effect on gK at low concentrations (<5 mM) and the potentiating effect on contractile response to various stimuli in the same range of concentrations.

We thus attempted to elucidate whether mechanical responses to various stimuli which depolarize the cell membrane would be potentiated by application of TEA, even at low concentrations, via its blocking action on the gK. Effects of TMA and TPA on K permeability and on mechanical activity were also examined in comparison with these factors of TEA to determine whether or not the quaternary ammonium ions play a direct role in Ca utilization.

Materials and Methods

The dissection of the strips from the canine trachea and measurement of tension were as described in a previous paper (9).

1. Electrical and mechanical stimulation: The muscle was passed through a pair of platinum ring electrodes for field stimulation. The diameter of the ring and the distance between the rings were 3 mm and 15 mm, respectively. Direct stimulation was carried out by a train of 20 square pulses of 10 msec duration at a frequency of 3 Hz instead of a single pulse of long duration because the magnitude of contraction was irregular when a single pulse at a duration above 10 msec was applied for direct electrical stimulation in the presence of TTX (0.5 μg/ml). The intensity of the stimulation was 10–15 V/cm, and the interval between the trains was 5 or 10 min. The stimulator used was model MSE-3 (Nihon Kohden Ltd.).

Mechanical stimulation was applied with a device of domestic make, as is illustrated in Fig. 1. To confirm that the myogenic response in the smooth muscle preparation could be validly measured using this apparatus, the effect of quick stretch on guinea pig taenia

![Fig. 1. A: Schematic diagram of the apparatus for quick stretch. The strip was loaded with 4.8 or 6.0 g of tension for 0.8 sec. B: A recording of the time course of quick stretch applied using this apparatus and without the muscle strip. C: Some traces of mechanical responses to quick stretch. See the text for evaluations of the mechanical response.](image-url)
coli which is known to demonstrate such a response was examined. We concluded that the apparatus was adequate for this purpose. The muscular tissues from canine trachea were carefully cut into segments, 1.5–2.0 mm in width and 19–21 mm in length. The strip was stretched by a transient elevation of load for 0.8 sec from 1 g (resting load) to 4.8 or 6.0 g. The rebound was absorbed by liquid paraffin. After a preincubation for 1 hr, the recorded contractile response to 40 mM K served as the control response. The magnitude of the contraction was between 9.6 g to 16.8 g (11.3±0.77 g, mean±S.E.M. of 14 observations). Quick stretch was then applied every 15 min except when the muscle contracted spontaneously in the presence of TEA. After a control stretch (4.8 g load), the length of the strip loaded with 1 g (resting load) ranged from 2.40 cm to 2.81 cm (2.61±0.11 cm, 14 observations). When the potassium concentration of the medium was elevated and/or quaternary ammonium salts were added, the mean amplitude of the responses was obtained from three trials of quick stretch. The relative amplitude of myogenic response (MR) was calculated from the equation: MR(%) = T(g) x 100/ [contractile response to 40 mM K], where T is the absolute value of the mechanical response. When TEA produced a spontaneous phasic contraction, quick stretch was applied after the muscle tone had decreased to the resting level.

2. Physiological solutions: A physiological solution of the following composition was used as a normal solution (mM): Na+, 137; K+, 5.9; Mg2+, 1.2; Ca2+, 2.2; Cl−, 123.5; HCO3−, 25; H2PO4−, 1.2; glucose, 14. This solution was equilibrated with 95% O2 and 5% CO2. The pH was 7.4–7.5 at 37°C. Solutions containing elevated concentrations of K were made by replacing an equivalent amount of sodium chloride with potassium chloride. In Ca-free solution, CaCl2 was omitted. In ion-flux experiments, an equivalent amount of sodium chloride was replaced with TEA and other quaternary ammonium chlorides. Chloride-deficient solutions were prepared by replacing chloride salts with corresponding acetate salts, except for the quaternary ammonium chloride. In one series of experiments, the Cl− concentration in the solution was kept constant. All solutions for ion-flux experiments contained 1.0 μM atropine.

3. Concentration-response curve for CaCl2: At first, a control response to isotonic 40 mM K solution was recorded. After washing for 30 min, the strip was kept in Ca-free solution containing 0.5 mM EGTA for 30 min. Then, the solution was replaced by Ca-free high K (111 mM) solution containing TEA or other drugs but without EGTA. After 10 min, CaCl2 was added cumulatively up to 8 mM. All solutions used in this experiment contained 3 μM atropine.

4. Measurements of 86Rb-efflux: The effect of three quaternary ammonium ions on K permeability in the tracheal smooth muscle was examined by using 86Rb as a tracer for K. In normal solution, TEA increased the efflux rate following the initiation of spike potentials, oscillations, and depolarization (12). For detection of the blocking effect of TEA on K permeability, experiments should be carried out on depolarized muscles. As the rate of both 42K and 86Rb-effluxes were too high and did not remain constant in high K solution, a prolonged experiment could not be carried out (12–14). When chloride ions in the efflux medium were replaced with large anions such as acetate ions, the rate of the 86Rb-efflux from depolarized tracheal smooth muscle could be kept constant. The experimental conditions and procedures were the same as those used elsewhere (12).

5. Drugs: The following drugs were used: atropine sulfate (Tokyo-Kasei Co. Ltd.), D-600 hydrochloride (Knoll A.G.), thiopen-
tone sodium (Tanabe Co. Ltd.), glycol-etherdiamine-\(\text{N},\text{N},\text{N}',\text{N}'\)-tetraacetic acid (EGTA) sodium, tetraethylammonium chloride (TEA), tetramethylammonium chloride (TMA) tetrapropylammonium chloride (TPA) (Wako Pure Chemicals).

6. Statistical methods: Most of the results were expressed as the mean±S.E.M. The Student's t-test or paired t-test (P=0.05 or 0.01) was used to determine statistical differences.

Results

1. Effect of TEA on myogenic response to quick stretch: Canine tracheal strips failed to respond actively to quick stretch under normal conditions, but did respond to stretch with only an increase in passive tension (Fig. 2). A myogenic response was, however, observed in the strip treated with TEA at concentrations over 10 mM, as reported by Stephens et al. (7). Figure 2C shows that high concentrations of TEA (20 and 30 mM) produced repetitive responses to a single stretch. Applications of TMA or TPA did not lead to a myogenic response, regardless of the concentration.

It should be noted that the concentration of TEA required to produce the myogenic response was low (~3 mM) in high K (10.9 or 15.9 mM) solution, as shown in Fig. 2A and B. Treatment with 10.9 mM K solution per se did not induce tension development, and a myogenic response to
quick stretch was not produced. In the same preparation, the threshold concentration of TEA necessary to produce a myogenic response seemed to lie between 10 and 15 mM in normal solution. High K solution per se failed to produce a myogenic response, at any concentration and in the absence of TEA. The effects of TEA on the response to quick stretch in normal and 10.9 mM K solution are summarized in Fig. 3. The myogenic response to quick stretch in the presence of TEA was sensitive to \([\text{Ca}]_o\) and was completely suppressed by Ca-deprivation from the medium or by an application of a Ca-blocker, 0.3 \(\mu\text{M} \text{ D-600} \) (not shown).

2. Effect of TEA, TMA and TPA on the contractile response to direct electrical stimulation: Figure 4 illustrates the effect of TMA, TEA, and TPA on the contractile response to direct electrical stimulation in the presence of 3 \(\mu\text{M} \text{ atropine}\). The contraction induced by the stimulation was almost completely inhibited by 1 \(\mu\text{M} \text{ D-600} \) or 3 \(\text{mM MnCl}_2\). Potentiation of the twitches by TEA occurred even at a concentration of less than 1 mM. As spontaneous contraction was initiated following the contraction induced by direct electrical stimulation, the effect of TEA on a contractile response to the stimulation could not be evaluated when the concentrations were over 8 mM. In most preparations, spontaneous rhythmic contractions were produced by TEA without electrical stimulation at concentrations over 15 mM (9). TMA (1–40 mM) did not induce mechanical activity in the presence of 1 \(\mu\text{M} \text{ atropine}\). A gradual increase in muscle tone was observed with the application of 5–30 mM TPA.

3. Effects of TMA, TEA and TPA on contractile response to isotonic high K solution: Cumulative dose response curves for potassium were determined in the presence or absence of 2 mM TEA (Fig. 5A). All solutions used contained 1 \(\mu\text{M} \text{ atropine}\). The curves are plotted as percentages of the maximum response to potassium in the absence of TEA. TEA significantly enhanced the response to 20 mM K, while it tended to decrease the maximum response to 40 mM K in the presence of Ca. After removal of TEA, the dose response curve for potassium came close to the control level. As a sustained contraction with an amplitude of approximately 70% of the maximum response to high K solutions was induced even in high K and Ca omitted solution (not shown), strips were incubated in a Ca free solution containing 0.5 mM EGTA for 20 min prior to the application of the solution to examine the effect of TEA on the response to high K and Ca free solutions. The enhancement by 2 mM TEA was not observed in Ca-free solutions. TMA (4 mM) did not significantly affect the dose response curve, and TPA (4 mM) decreased the maximum response by approximately 50% (Fig. 5B). TMA and TPA did not cause a TEA-like potentiation of the response to 20 mM K.

4. Effect of TEA on the contractile response to \(\text{CaCl}_2\) in a high K solution: The contractile response to \(\text{CaCl}_2\) of the depolarized tracheal
smooth muscle at concentrations of Ca from 0.1 to 8.0 mM and the effect of TEA on this response were examined. TEA at concentrations of 2 and 4 mM slightly shifted the dose-response curve for CaCl₂ to the right at the Ca concentrations of 0.05, 0.1, 0.3, 1.0, 2.0 and 4.0 mM; but the difference was not statistically significant (P>0.1, N=4–9). The response to 8 mM Ca was not affected by 2 and 4 mM TEA at all.
5. Effect of TMA and TPA on $^{86}$Rb-efflux from depolarized muscle: In previous work, we found that TEA decreased the $^{86}$Rb-efflux from depolarized tracheal smooth muscle in a concentration dependent manner (12). Thus, the effects of TMA and TPA on K permeability were examined under the same conditions (see Methods). Figure 6A illustrates the effect of 10 mM TPA on $^{86}$Rb-efflux in high K acetate solution and the method for evaluation. Summarized results of the effects of three ammonium ions on $^{86}$Rb-efflux are shown in Fig. 6B. TMA did not produce a significant decrease at any concentration up to 30 mM. Although TPA decreased the rate of efflux significantly at high concentrations, TEA was far more effective than TPA.

Discussion

Pharmacological approaches have been used to investigate TEA-induced potentiation of contractile responses to various agonists in smooth muscle. Two possible mechanisms have been suggested: one is that TEA acts directly on Ca mobilization as a quaternary ammonium ion and increases the utilization of extracellular and/or superficially bound calcium for contraction (10), and another is that TEA potentiates the depolarization induced by agonists because of the blockade of gK and produces a consequent increase in the influx of Ca (15–17). Although the potentiating effect of TEA at concentrations less than 1 mM has been known (10, 11), high concentrations of TEA ($\geq 10$ mM) were used in the studies suggesting the latter mechanism.

In previous work (12), we found that TEA blocks the K permeability of the depolarized cell membrane of canine tracheal smooth muscle at low concentrations (KD value $\approx 1$ mM). The relationship between the K permeability in high K solutions monitored by $^{42}$K or $^{86}$Rb-efflux and the gK, which is detected in voltage clamp studies and probably functioning in the falling phase of action potential (18–21), has remained undetermined. The former was decreased by TEA at relatively low concentrations ($\leq 5$ mM), while the latter is decreased at higher concentrations of TEA (18, 20). Although the difference in sensitivity of the gK(s) and the K permeability to TEA can not be explained now, it can be postulated that the gK(s) is(are) well activated and becomes (or become) more sensitive to TEA in depolarized and contracted smooth muscle in high K solution. However, some investigators have reported that TEA, at low concentrations, decreases the rectification to depolarizing current and increases the excitability without affecting resting membrane potential (2–5 mM in stomach smooth muscle of the guinea pig (22) and the dog (23); 5 mM in dog trachea (8)). The present finding that TEA potentiated the contractile response to direct electrical stimulation at low concentrations are in good agreement with these results. Thus, we suppose that TEA blocks the gK(s) at low concentrations and increases the amplitude of depolarization by direct electrical stimulation and that this results in the increase in transmembrane Ca influx in canine tracheal smooth muscle.

The same mechanism is likely to be involved in the myogenic response to quick stretch. It has been reported that quick stretch depolarizes the cell membrane and elicits a burst of spikes resulting in a myogenic response in the taenia coli of guinea-pig (24) and that the response is activated almost entirely by Ca ion derived from the extracellular medium (7). As an occurrence of the response to quick stretch is indicative of the excitation-contraction coupling in a muscle, the method is quite useful for investigating the relationship between membrane excitability and contractile activity of smooth muscle (7, 25, 26). When the
tracheal muscle strip was quickly stretched in the presence of TEA, the transient depolarization and following spike potentials may elicit the myogenic response (7). In 10.9 mM K solution, the myogenic responses were observed even when the concentration of TEA was one third or one fifth of that required for induction in normal solution. This finding indicates that a low concentration of TEA was sufficient to produce the myogenic response if the membrane was depolarized near threshold by some means. Under similar conditions (15.9 mM K and 5 mM TEA), spontaneous electrical activities such as oscillations and spikes were observed in most preparations, while 5 mM TEA by itself rarely produced spontaneous activity in normal solution (12).

The membrane potential in high K solution was, however, much less than that in the absence of TEA, particularly when the K concentrations were relatively low (12, 16). This is in good agreement with the findings that the contraction induced by 20 mM K solution was significantly potentiated by 2 and 4 mM TEA. On the other hand, TEA in concentrations of 2 and 4 mM slightly decreased the contractile response to CaCl₂ in strongly depolarized muscle. These findings indicate that low concentrations of TEA increase the transmembrane Ca-influx following an increase in depolarization of the membrane.

TEA decreased the rate coefficient of ⁸⁶Rb efflux by 27% at a concentration of 1 mM (approximate KD value), while TMA and TPA had little effect even at higher concentrations. These findings indicate the high selectivity of the molecular volume and structure of TEA as a blocker of potassium permeability in the depolarized tracheal smooth muscle cell membrane. It has been assumed that the ethyl groups around the charged nitrogen which give this portion of the molecule a diameter of about 8Å are important for the activity in blocking the K channel in squid giant axon and Ranvier's node because the diameter corresponds to that of K ions hydrated with the water molecules (27–30). In keeping with the idea that the depolarization induced by stimuli is potentiated by the decrease in gK, the lack of effect of TMA and TPA on K permeability is in good agreement with the findings that these two ammonium ions failed to potentiate any contractile responses examined in this study.

TEA (2 and 4 mM) did not affect significantly the contractile responses to Ca in depolarized muscle, while TPA (2 mM) significantly decreased the response to high K. These findings indicate that TPA may have direct effect on Ca mobilization and rather decrease Ca utilization in smooth muscle, but TEA does not. Though it was suggested that TEA, as a quaternary ammonium ion, might have an direct action on calcium mobilization and increase the utilization of extracellular and/or superficially bound calcium for contraction during agonist action (10), no evidence supporting this suggestion was obtained in our results. Such a possibility should be reexamined with regard to the effects of TMA, TEA and TPA on contractile responses via drug-receptor interactions.

In conclusion, the results in this study indicate that TEA decreases gK(s) at low concentrations (<5 mM) and may increase the stimuli-induced depolarization, resulting in the potentiation of the transmembrane Ca-influx.

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