Electrical behavior of guinea pig tracheal smooth muscle

NARELLE J. BRAMICH
Department of Zoology, University of Melbourne, Parkville, Victoria 3052, Australia

Bramich, Narelle J. Electrical behavior of guinea pig tracheal smooth muscle. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L320–L328, 2000.—Intracellular recordings were taken from the smooth muscle of the guinea pig trachea, and the effects of intrinsic nerve stimulation were examined. Approximately 50% of the cells had stable resting membrane potentials of −50 ± 1 mV. The remaining cells displayed spontaneous oscillations in membrane potential, which were abolished either by blocking voltage-dependent Ca2+ channels with nifedipine or by depleting intracellular Ca2+ stores with ryanodine. In quiescent cells, stimulation with a single impulse evoked an excitatory junction potential (EJP). In 30% of these cells, trains of stimuli evoked an EJP that was followed by oscillations in membrane potential. Transmural nerve stimulation caused an increase in the frequency of spontaneous oscillations. All responses were abolished by the muscarinic-receptor antagonist hyoscine (1 µM). In quiescent cells, nifedipine (1 µM) reduced EJPs by 30%, whereas ryanodine (10 µM) reduced EJPs by 93%. These results suggest that both the release of Ca2+ from intracellular stores and the influx of Ca2+ through voltage-dependent Ca2+ channels are important determinants of spontaneous and nerve-evoked electrical activity of guinea pig tracheal smooth muscle.

excitatory junction potential

MAMMALIAN AIRWAY SMOOTH MUSCLE receives a parasympathetic excitatory innervation originating from the vagus. In most species, stimulation of vagal nerve fibers evokes a contraction of tracheal smooth muscle that is abolished by the muscarinic-receptor antagonist atropine, indicating that acetylcholine (ACh) is released from postganglionic nerve fibers, causing the activation of muscarinic receptors (7). Contraction evoked by parasympathetic nerve stimulation is preceded by a membrane depolarization or excitatory junction potential (EJP). Studies examining the effect of dihydropyridine Ca2+ antagonists on responses evoked by parasympathetic nerve stimulation suggest that EJPs and contractions are partly mediated by the influx of Ca2+ through voltage-dependent L-type Ca2+ (CaL) channels (11, 21, 31). In contrast, studies have shown that responses to exogenously applied ACh are little affected by either dihydropyridine Ca2+ antagonists or low external Ca2+ (1, 2, 13, 21, 26, 27). This has led to the suggestion that the influx of Ca2+ through CaL channels has little importance in the generation of tracheal smooth muscle contraction evoked by muscarinic-receptor stimulation. Instead, it has been proposed that muscarinic-receptor stimulation increases the intracellular concentration of Ca2+ after either the release of Ca2+ from intracellular stores via an inositol 1,4,5-trisphosphate-dependent process (see Ref. 4) or the entry of Ca2+ through receptor-operated Ca2+ channels (33).

The difference in effectiveness of Ca2+ antagonists to reduce responses to exogenously applied and neurally released ACh in tracheal smooth muscle might suggest that the two sources of transmitter activate distinct receptors, bringing about different ionic changes. A difference in the effects of a neurally released and exogenously applied transmitter has been observed at a number of neuroeffector junctions in the autonomic nervous system (see Ref. 16). For example, in guinea pig ileal smooth muscle different sources of Ca2+ are responsible for the membrane depolarizations and contractions evoked by exogenously applied and neurally released ACh (8, 9). Although the ionic mechanisms underlying the responses to exogenously applied ACh in tracheal smooth muscle have been extensively studied, it is unclear whether the same mechanisms can account for the responses evoked by parasympathetic nerve stimulation (11, 31). The present study tested the hypothesis that nerve-evoked responses of tracheal smooth muscle cells depend on both release of Ca2+ from intracellular stores and the influx of Ca2+ through CaL channels.

METHODS

The procedures described have been approved by the Animal Experimentation Ethics Committee at the University of Melbourne (Parkville, Australia). Guinea pigs of either sex, weighing 150–200 g, were killed by a blow to the head and exsanguination. A segment of trachea containing four to five cartilaginous rings was taken from just above the bifurcation of the two bronchi. A cut was made through the cartilage down the length of the trachea, and the epithelium along with any visible connective tissue was stripped from the muscle. Preparations were pinned in a shallow recording chamber (bath volume 2 ml) with pins cut from 100-µm tungsten wire. The base of the recording chamber consisted of a coverslip coated with Sylgard silicone resin (Dow Corning, Midland, MI). The preparations were placed over a platinum electrode, and a second platinum electrode was placed in the bath to allow stimulation of intrinsic nerve fibers (1–90 V, 0.1–1.0 ms). Preliminary experiments indicated that supramaximal responses could be obtained with pulse widths of 0.5 ms and amplitudes of 80–90 V. These stimulus parameters were used in all experiments. The preparations were continuously perfused with physiological saline (composition in mM: 119.8 NaCl, 5.0 KCl, 25 NaHCO3, 1.0 NaH2PO4, 2.5 CaCl2, 2.0
MgCl₂, and 11 glucose, gassed with 95% O₂-5% CO₂ at a rate of 3 ml/min. Unless otherwise stated, experiments were performed in the presence of propranolol (1 µM) and indomethacin (10 µM). The drugs were added to the preparation by changing the inflow line from the control solution to one containing the appropriate concentration of drug. All experiments were performed at 35°C.

Intracellular recordings were made from the epithelial side of the tissue with the use of conventional techniques with fine glass microelectrodes (resistance 100–210 MΩ) filled with 0.5 M KCl. All membrane potential records were low-pass filtered (cutoff frequency 1 kHz), digitized (100 Hz), and stored on disk for later analysis. At the start of each experiment, several successive recordings were made from each tissue, and the myogenic behavior of the tissue was characterized. Subsequently, the tissues were stimulated transmurally with a single impulse or trains of impulses at 2-min intervals. At least three consecutive EJPs with the same amplitude and time course were obtained before the addition of any drug. Control measurements were taken from the last EJP recorded before the addition of a drug. Measurements were taken from a single EJP after responses had reached a new steady state after drug treatment (after an equilibration time of at least 30 min). Due to variation in the amplitude of responses recorded from different cells, in any particular experiment all responses were recorded from the same cell. If an impalement was lost during the course of an experiment, these results were discarded. Latencies of EJPs were measured from the stimulus artifact to 10% of the peak amplitude. Rise times were measured from 10 to 90% of the peak amplitude. Half-widths were measured as the time between 50% of the peak amplitude on the rising and falling phases of the EJPs. All values are expressed as means ± SE. Each n value represents a measurement from a different animal. Where indicated, the significance of the difference between two means was determined with Student’s t-test. To eliminate any possible effect of prostaglandins on nerve-evoked responses, experiments were performed in the presence of indomethacin (10 µM).

The drugs used in this study were nifedipine hydrochloride, hyoscine sulfate, tetrodotoxin, caffeine, isethionate, tetraethylammonium chloride, barium chloride, indomethacin, propranolol (all from Sigma, St. Louis, MO), and ryanodine (Calbiochem, Alexandria, Australia). All drugs were dissolved in distilled water except nifedipine and indomethacin, which were dissolved in absolute ethanol.

RESULTS

General observations. Intracellular recordings taken from guinea pig tracheal smooth muscle displayed three types of electrical activity. In all preparations, if sufficient different bundles of tracheal muscle were impaled, spontaneous oscillations in membrane potential were detected (Fig. 1, B and C). The oscillations had an amplitude of 25.6 ± 1.3 mV (n = 20) and a peak negative potential around −50 mV. The frequency of the membrane potential oscillations ranged between 11 and 44 cycles/min (mean 23.7 ± 1.9 cycles/min; n = 20). In some cells, an action potential spike was superimposed on the peak of each oscillation (Fig. 1C). In each preparation, the oscillations detected from successive bundles had similar properties. In 80% of the preparations, it was possible to record from a bundle of tracheal muscle in which the cells were quiescent; these had a stable resting membrane potential of −50 ± 1 mV (n = 20) and 11 glucose, gassed with 95% O₂-5% CO₂ at a rate of 3 ml/min. Unless otherwise stated, experiments were performed in the presence of propranolol (1 µM) and indomethacin (10 µM). The drugs were added to the preparation by changing the inflow line from the control solution to one containing the appropriate concentration of drug. All experiments were performed at 35°C.

Intracellular recordings were made from the epithelial side of the tissue with the use of conventional techniques with fine glass microelectrodes (resistance 100–210 MΩ) filled with 0.5 M KCl. All membrane potential records were low-pass filtered (cutoff frequency 1 kHz), digitized (100 Hz), and stored on disk for later analysis. At the start of each experiment, several successive recordings were made from each tissue, and the myogenic behavior of the tissue was characterized. Subsequently, the tissues were stimulated transmurally with a single impulse or trains of impulses at 2-min intervals. At least three consecutive EJPs with the same amplitude and time course were obtained before the addition of any drug. Control measurements were taken from the last EJP recorded before the addition of a drug. Measurements were taken from a single EJP after responses had reached a new steady state after drug treatment (after an equilibration time of at least 30 min). Due to variation in the amplitude of responses recorded from different cells, in any particular experiment all responses were recorded from the same cell. If an impalement was lost during the course of an experiment, these results were discarded. Latencies of EJPs were measured from the stimulus artifact to 10% of the peak amplitude. Rise times were measured from 10 to 90% of the peak amplitude. Half-widths were measured as the time between 50% of the peak amplitude on the rising and falling phases of the EJPs. All values are expressed as means ± SE. Each n value represents a measurement from a different animal. Where indicated, the significance of the difference between two means was determined with Student’s t-test. To eliminate any possible effect of prostaglandins on nerve-evoked responses, experiments were performed in the presence of indomethacin (10 µM).

The drugs used in this study were nifedipine hydrochloride, hyoscine sulfate, tetrodotoxin, caffeine, isethionate, tetraethylammonium chloride, barium chloride, indomethacin, propranolol (all from Sigma, St. Louis, MO), and ryanodine (Calbiochem, Alexandria, Australia). All drugs were dissolved in distilled water except nifedipine and indomethacin, which were dissolved in absolute ethanol.

RESULTS

General observations. Intracellular recordings taken from guinea pig tracheal smooth muscle displayed three types of electrical activity. In all preparations, if sufficient different bundles of tracheal muscle were impaled, spontaneous oscillations in membrane potential were detected (Fig. 1, B and C). The oscillations had an amplitude of 25.6 ± 1.3 mV (n = 20) and a peak negative potential around −50 mV. The frequency of the membrane potential oscillations ranged between 11 and 44 cycles/min (mean 23.7 ± 1.9 cycles/min; n = 20). In some cells, an action potential spike was superimposed on the peak of each oscillation (Fig. 1C). In each preparation, the oscillations detected from successive bundles had similar properties. In 80% of the preparations, it was possible to record from a bundle of tracheal muscle in which the cells were quiescent; these had a stable resting membrane potential of −50 ± 1 mV (n = 20) and 11 glucose, gassed with 95% O₂-5% CO₂ at a rate of 3 ml/min. Unless otherwise stated, experiments were performed in the presence of propranolol (1 µM) and indomethacin (10 µM). The drugs were added to the preparation by changing the inflow line from the control solution to one containing the appropriate concentration of drug. All experiments were performed at 35°C.

Intracellular recordings were made from the epithelial side of the tissue with the use of conventional techniques with fine glass microelectrodes (resistance 100–210 MΩ) filled with 0.5 M KCl. All membrane potential records were low-pass filtered (cutoff frequency 1 kHz), digitized (100 Hz), and stored on disk for later analysis. At the start of each experiment, several successive recordings were made from each tissue, and the myogenic behavior of the tissue was characterized. Subsequently, the tissues were stimulated transmurally with a single impulse or trains of impulses at 2-min intervals. At least three consecutive EJPs with the same amplitude and time course were obtained before the addition of any drug. Control measurements were taken from the last EJP recorded before the addition of a drug. Measurements were taken from a single EJP after responses had reached a new steady state after drug treatment (after an equilibration time of at least 30 min). Due to variation in the amplitude of responses recorded from different cells, in any particular experiment all responses were recorded from the same cell. If an impalement was lost during the course of an experiment, these results were discarded. Latencies of EJPs were measured from the stimulus artifact to 10% of the peak amplitude. Rise times were measured from 10 to 90% of the peak amplitude. Half-widths were measured as the time between 50% of the peak amplitude on the rising and falling phases of the EJPs. All values are expressed as means ± SE. Each n value represents a measurement from a different animal. Where indicated, the significance of the difference between two means was determined with Student’s t-test. To eliminate any possible effect of prostaglandins on nerve-evoked responses, experiments were performed in the presence of indomethacin (10 µM).

The drugs used in this study were nifedipine hydrochloride, hyoscine sulfate, tetrodotoxin, caffeine, isethionate, tetraethylammonium chloride, barium chloride, indomethacin, propranolol (all from Sigma, St. Louis, MO), and ryanodine (Calbiochem, Alexandria, Australia). All drugs were dissolved in distilled water except nifedipine and indomethacin, which were dissolved in absolute ethanol.

RESULTS

General observations. Intracellular recordings taken from guinea pig tracheal smooth muscle displayed three types of electrical activity. In all preparations, if sufficient different bundles of tracheal muscle were impaled, spontaneous oscillations in membrane potential were detected (Fig. 1, B and C). The oscillations had an amplitude of 25.6 ± 1.3 mV (n = 20) and a peak negative potential around −50 mV. The frequency of the membrane potential oscillations ranged between 11 and 44 cycles/min (mean 23.7 ± 1.9 cycles/min; n = 20). In some cells, an action potential spike was superimposed on the peak of each oscillation (Fig. 1C). In each preparation, the oscillations detected from successive bundles had similar properties. In 80% of the preparations, it was possible to record from a bundle of tracheal muscle in which the cells were quiescent; these had a stable resting membrane potential of −50 ± 1 mV (n = 20).
The membrane potential of such cells was interrupted by brief membrane depolarizations with amplitudes up to 5 mV (Fig. 1A). In 35% of the preparations, a second type of rhythmic activity was detected in 20% of the tracheal bundles. These cells generated bursts of spontaneous activity that were interspersed with quiescent periods of 1–4 min in duration (Fig. 1D). In these cells, the resting membrane potential during periods of quiescence was −45 to −60 mV (mean −53.4 ± 4.0 mV; n = 5). A gradual depolarization of the resting membrane potential preceded each burst of activity.

Indomethacin (1–10 µM) had no effect on the frequency or amplitude of membrane potential oscillations (n = 5; Fig. 2A). Oscillations ceased in the presence of nifedipine (10 µM), and the membrane potential settled near −50 mV (n = 10; Fig. 2B). Depletion of intracellular stores with ryanodine (10 µM) abolished the oscillations (Fig. 2C). Ryanodine initially depolarized the smooth muscle by −30 mV before the depolarization settled at a value of −43 ± 4 mV (n = 5) after ~1 h.

Membrane potential changes evoked by intrinsic nerve stimulation. In quiescent bundles of tracheal muscle, stimulation of intrinsic nerve fibers with a single impulse evoked an EJP (Fig. 3, Aa and Ba). EJPs had amplitudes of 11.5–35.0 mV (mean 24.4 ± 1.4 mV), latency of 215 ± 12 ms, rise time of 246 ± 24 ms, and half-width of 525 ± 23 ms (n = 26). After a peak was reached, the membrane potential rapidly repolarized to a value close to the resting membrane potential and then gradually returned to the control level over the next 15–20 s (Fig. 3Ba). Usually, the slowly decaying phase of the EJP appeared as a separate component, with a peak amplitude of 2.7 ± 0.4 mV (n = 20; Fig. 3, Aa; see also Fig. 5A).

Increasing the number of stimuli (1–10 impulses at 10 Hz) did not change the peak amplitude of the EJPs. However, in 30% of the cells recorded from, multiple stimuli triggered large fluctuations in membrane potential (Fig. 3A, b and c). A further increase in the number of stimuli resulted in the initiation of oscillations that lasted for some 20–60 s (Fig. 3Ad). In the other quiescent cells (70%), increased numbers of stimuli often initiated a second rapid phase and increased the amplitude of the slow secondary phase of the EJP (Fig. 3B, b and c). In these cells, stimulation with 10 impulses at 10 Hz evoked an initial depolarization of 26.2 ± 2.0 mV, a secondary depolarization of 19.3 ± 3.8 mV, and a slow membrane depolarization of 6.0 ± 0.8 mV (n = 11; Fig. 3Bd); further increases in the number of stimuli failed to initiate oscillations in membrane potential.

![Fig. 2. Effect of indomethacin, nifedipine, and ryanodine on spontaneous membrane potential oscillations recorded from tracheal smooth muscle of guinea pig.](https://example.com/fig2.png)
In spontaneously active cells, a single nerve stimulus had little effect on either the frequency or amplitude of the membrane potential oscillations (Fig. 4A). However, increasing the number of stimuli increased the frequency of the membrane potential oscillations for some 10 s after the period of stimulation. Thus 10 impulses at 10 Hz increased the frequency of the oscillations from 27 ± 4 to 48 ± 5 cycles/min (n = 7; Fig. 4B).
During this time, the peak negativity of the membrane potential decreased by 6.3 ± 1.9 mV. This effect of nerve stimulation on rhythmic activity was more evident with lower frequencies of stimulation (2 Hz, 5 s; Fig. 4C).

The membrane potential changes evoked by transmural nerve stimulation resulted solely from the stimulation of parasympathetic nerve fibers and release of ACh because all responses were abolished after the addition of the muscarinic-receptor antagonist hyoscine (1 µM) to the physiological saline (n = 6). Both the spontaneous oscillations in membrane potential and the small spontaneous membrane depolarizations persisted with hyoscine.

The observation that, in any given preparation, three electrically different cell types could be recorded from suggests that either the behavior of the preparations changes with time or the cells within any given preparation may display quite different electrical properties. To distinguish between these two possibilities, pairs of recordings were made simultaneously from nearby smooth muscle cells in the same muscle bundle or from smooth muscle cells in neighboring muscle bundles. Pairs of cells impaled in the same muscle bundle, which originated from same cartilaginous ring, had similar electrical characteristics and responses to intrinsic nerve stimulation (n = 3; Fig. 5, A and B). However, if simultaneous recordings were made from muscle bundles that originated from different cartilaginous rings, the electrical responses were not synchronized (n = 3). For example, in a pair of quiescent cells, stimulation of the parasympathetic nerves with a single impulse evoked an EJP that had a similar amplitude and time course in both cells (Fig. 5C). However, short trains of stimuli evoked oscillations in one cell but not in the other (Fig. 5D).

Sources of Ca^{2+} responsible for the generation of EJPs. Nifedipine (1–10 µM) reduced EJPs by ~30% (Fig. 6). In the control solution, the EJPs evoked by a single impulse had an amplitude of 23.1 ± 2.3 mV. With

---

**Fig. 5.** Simultaneous recordings of membrane potential taken from cells in the same and different muscle bundles of guinea pig trachea. A: recordings taken from 2 cells in the same muscle bundle. Stimulation with a single impulse evoked an EJP that was followed by initiation of membrane potential oscillations. Resting membrane potentials in Aa and Ab were −49 and −50 mV, respectively. B: 2 recordings made simultaneously from cells within the same muscle bundle. Spontaneous oscillations, which were recorded from both cells, were synchronous and of similar amplitude. Stimulation of intrinsic nerves with a single impulse caused a similar membrane potential change in both cells. Peak negative potential in B was around −49 mV. C and D: 2 simultaneous membrane potential recordings taken from cells in neighboring muscle bundles of trachea. Stimulation of intrinsic nerves evoked an EJP in both cells that had similar amplitudes and time courses. Resting membrane potentials in C, a and b, were −45 and −51 mV, respectively. When stimulus number was increased to 5 impulses delivered at 10 Hz, membrane potential oscillations were initiated in one cell (Da) but not in the 2nd cell (Db). Indomethacin (10 µM) was present throughout.
nifedipine (1 µM), the EJPs were reduced to 15.9 ± 2.6 mV (P < 0.05; n = 12). The slowly decaying component of the EJP was also inhibited by nifedipine (Figs. 6 and 7); with control physiological saline, the peak depolarization was 2.6 ± 0.6 mV, and after the addition of nifedipine, the depolarization was 0.8 ± 1.0 mV (P < 0.05; n = 12). Increasing the concentration of nifedipine (10 µM) did not further reduce the amplitude of EJPs (n = 3). Nifedipine abolished oscillations triggered by cholinergic nerve stimulation to reveal EJPs similar to those seen in quiescent cells (Fig. 6 B, b and c).

Ryanodine (10 µM) caused a 93% reduction in the peak amplitude (control, 26.5 ± 2.4 mV; ryanodine, 2.3 ± 1.2 mV; n = 5) and abolished the slowly decaying phase of EJPs evoked by single stimuli (Fig. 7B, a and b). Caffeine (3 mM) abolished the EJPs evoked by a single impulse (n = 3). Ryanodine or caffeine abolished the nifedipine-resistant small spontaneous depolarizations. In preparations pretreated with nifedipine (10 µM), ryanodine (10 µM, 60 min) reduced the amplitude of EJPs from 15.1 ± 2.3 to 4.0 ± 1.6 mV (P < 0.05; n = 7; Fig. 7Ac). However, if preparations were pretreated with ryanodine (10 µM), the subsequent addition of nifedipine had no effect on ryanodine-resistant EJPs (Fig. 7Bc). The EJPs had amplitudes of 29.0 ± 2.2 and 3.7 ± 1.3 mV in the control and ryanodine-containing solutions, respectively. After nifedipine was added, the EJPs had an amplitude of 3.8 ± 1.6 mV (n = 3).

The small nerve-evoked depolarizations that persisted in the presence of both nifedipine and ryanodine had time courses similar to the control EJPs. This resistant component increased in amplitude with an increasing number of stimuli and was invariably abolished by hyoscine (1 µM; n = 3; Fig. 7, Ad and Bd).

**DISCUSSION**

The present study has shown that the responses to cholinergic nerve stimulation in tracheal smooth muscle result both from the release of Ca²⁺ from intracellular stores and from the entry of Ca²⁺ via CaL channels. After release from the intracellular stores, Ca²⁺ activates sets of Ca²⁺-activated Cl⁻ channels to trigger a depolarization, which, in turn, activates CaL channels. In addition, many preparations generate spontaneous...
myogenic activity that also appears to result from intracellular Ca\textsuperscript{2+} release and the influx of Ca\textsuperscript{2+} through Ca\textsubscript{L} channels.

Few studies have indicated a role for Ca\textsuperscript{2+} entry through Ca\textsubscript{L} channels in the generation of agonist-induced contractions (10, 15), with Ca\textsuperscript{2+} antagonists or a low external Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) having little effect on the responses evoked by muscarinic-receptor agonists (1, 2, 13, 21, 26, 27). The insensitivity of agonist-induced contractions to Ca\textsuperscript{2+} antagonists has
led to the suggestion that contraction of airway smooth muscle results solely from intracellular Ca$^{2+}$ release after the production of inositol 1,4,5-trisphosphate (4). Membrane depolarization then results secondarily from an increase in intracellular [Ca$^{2+}$]_{i} and the subsequent activation of Ca$^{2+}$-dependent Cl$^{-}$ conductances (23, 24). Clearly, the role played by Ca$^{2+}$ entry through Ca$_{L}$ channels in the generation of tracheal smooth muscle contraction may vary depending on the source of ACh. In the present study, tracheal EJPs with characteristics similar to those previously described in guinea pig, dog, and ox airway smooth muscle (6, 13, 20, 30) were reduced but not abolished by nifedipine, suggesting that Ca$^{2+}$ entry through Ca$_{L}$ channels contributes to the membrane depolarization. EJPs were also greatly attenuated after the depletion of Ca$^{2+}$ from the intracellular stores with either caffeine or ryanodine. Because nifedipine reduced the amplitude of EJPs but not after Ca$^{2+}$ store depletion, an initial membrane depolarization must be required to initiate Ca$^{2+}$ entry through Ca$_{L}$ channels. In the present study, there was no evidence for the activation of receptor-operated Ca$^{2+}$ channels by parasympathetic nerve stimulation (see Ref. 33). Although a small membrane depolarization remained in the presence of ryanodine, this was abolished by a low [Cl$^{-}$]o, suggesting that it results from the release of Ca$^{2+}$ from inside the cells. Perhaps stores were not completely depleted by the addition of ryanodine or, alternatively, a second internal store may be involved in this component of the response. The latter suggestion is supported by the observation that caffeine, in contrast to ryanodine, was able to abolish EJPs.

Spontaneous electrical slow-wave activity has been observed in airway smooth muscle of the guinea pig (1, 17, 30, 32, 34), human (18), ox (28), and dog (21). Similar slow-wave activity may also be initiated by parasympathetic nerve stimulation (22), the bath application of ACh (6, 21), or blocking K channels with tetraethylammonium chloride (21). Both spontaneous and agonist-evoked slow waves were abolished after removal of external Ca$^{2+}$ and after Ca$_{L}$ channels were blocked with nifedipine (1, 3, 21, 27). It has therefore been suggested that Ca$^{2+}$ entry through Ca$_{L}$ channels is required for the generation of slow-wave activity in tracheal smooth muscle. In the present study, spontaneous and evoked membrane potential oscillations were abolished by nifedipine or after the depletion of intracellular Ca$^{2+}$ stores with ryanodine. Thus an increase in [Ca$^{2+}$]_{i}, resulting from either the influx of Ca$^{2+}$ through Ca$_{L}$ channels or the second messenger release of Ca$^{2+}$ from intracellular stores may be required for the initiation of Ca$^{2+}$-induced Ca$^{2+}$ release from the intracellular stores and the maintenance of membrane potential oscillations. In isolated guinea pig tracheal smooth muscle cells, spontaneous transient inward currents (STICs), which result from activation of Ca$^{2+}$-activated Cl$^{-}$ channels, display bursts of rhythmic activity that persist when the membrane potential is voltage-damped to \(-60\) mV to inhibit the influx of Ca$^{2+}$ through Ca$_{L}$ channels (25). It has therefore been suggested that STICs, which result from the cyclic release of Ca$^{2+}$ from intracellular stores, provide the basis for rhythmic activity (25). The oscillations in membrane potential and [Ca$^{2+}$]_{i} observed in tracheal smooth muscle might therefore result from the generation of STICs, causing membrane depolarization and the activation of Ca$_{L}$ channels. The influx of Ca$^{2+}$ through Ca$_{L}$ channels would then result in further release and reuptake of Ca$^{2+}$ from intracellular stores.

In the present experiments, it was apparent that different muscle bundles from within any given preparation often showed differing spontaneous activity and may respond differently to parasympathetic nerve stimulation. This suggests that smooth muscle bundles of the trachea are not well coupled. This has previously been implied in the guinea pig trachea where electrical and tension recordings taken simultaneously from two different regions of the trachea displayed quite different behaviors (14). This is despite the histological evidence that neighboring muscle bundles form cross-connections with one another (14, 19). Such poor coupling within muscle bundles of tracheal smooth muscle may explain the apparent discrepancies in observations in the actions of nifedipine on both spontaneous activity and the responses to cholinergic agonists in tracheal smooth muscle. For example, it has been suggested that membrane potential oscillations in tracheal smooth muscle are not directly representative of tone (1, 34). This is based on the observation that nifedipine abolishes oscillations in membrane potential but has no effect on overall tone of the tissue. If muscle bundles within the trachea act independently of one another, changes in membrane potential occurring in one region of the smooth muscle may not truly reflect the changes that are occurring in other regions of the smooth muscle. Therefore, although stimulation of the parasympathetic nerves appears to produce a synchronization of responses in all muscle bundles, the long-term effects of nerve stimulation may differ between muscle bundles. Presumably, this allows for both the local and overall regulation of tracheal smooth muscle tone.

I thank Prof. David Hirst and Dr. Frank Edwards for helpful discussions and comments on the manuscript. This work was supported by the National Health and Medical Research Council of Australia.

Address for reprint requests and other correspondence: N. J. Bramich, Dept. of Zoology, Univ. of Melbourne, Parkville, Victoria 3052, Australia (E-mail: n.j.bramich@zoology.unimelb.edu.au).

Received 28 December 1998; accepted in final form 10 September 1999.

REFERENCES


