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ACTIONS OF INDOMETHACIN AND PROSTAGLANDINS ON NEURO-EFFECTOR TRANSMISSION IN THE DOG TRACHEA

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SUMMARY

Neuro-effector transmission in the smooth muscle layer of the dog trachea was studied *in vitro* using the micro-electrode and double sucrose gap methods.

1. Electrical field stimulations with short duration (50–100 μsec) applied to the whole tissue produced an excitation of the intrinsic nerves, and evoked excitatory junction potentials (e.j.p.s) followed by twitch tension development and subsequent long lasting relaxation of the smooth muscle tissue.

2. The effects of field stimulations were abolished by tetrodotoxin (2×10^{-7} M), and atropine ($1{\cdot}7 \times 10^{-5}$ M) selectively blocked both the e.j.p. and twitch tension. On the other hand, propranolol ($1{\cdot}9 \times 10^{-5}$ M) suppressed the generation of the prolonged relaxation evoked by the field stimulations.

3. E.j.p.s recorded by the double sucrose gap method showed gradual and continuous reduction in amplitude during prolonged exposure in Krebs solution (1–2 hr), and there were no changes in the membrane potential or in the input membrane resistance.

4. With application of indomethacin (10^{-5} M), a gradual and continuous reduction in the amplitude of e.j.p. was no longer observed, and (after the initial increase in the amplitude) e.j.p.s with a constant amplitude were obtained during 1–1·5 hr. Indomethacin (10^{-5} M) modified neither the resting membrane potential nor the input membrane resistance of smooth muscle cells.

5. After pre-treatment with indomethacin, low concentrations (10^{-11}–10^{-8} M) of prostaglandin E_1 or E_2 (PGE series) markedly suppressed the amplitude of e.j.p. with no changes in the resting membrane potential or in the input membrane resistance.

6. During the repetitive field stimulation at the stimulus frequency of 0·1–1 Hz, the amplitude of the e.j.p.s was gradually reduced (the depression process). The depression was not affected by applications of prostaglandins, indomethacin or α- and β-adrenoceptor blockers.

7. These results indicate that in the dog tracheal smooth muscles, the endogenous PGE series may play an important role in feed-back inhibitory mechanisms, at the nerve terminals related to acetylcholine release.

INTRODUCTION

The tracheobronchial smooth muscle is considered to be controlled by the autonomic nervous system and there is recently reported evidence that non-adrenergic and non-cholinergic inhibitory nerves are present in respiratory airways in human and guinea-pigs (Coburn & Tomita, 1973; Richardson & Bouchard, 1975; Richardson & Beland, 1976; Richardson & Ferguson, 1979).

Although there is an abundance of information available on the nervous control of smooth muscle of the respiratory airway (see for example Widdicombe, 1963, 1966), relatively little is known of the properties of neuro-effector transmission or the regulatory mechanisms for transmitter release.

In an attempt to elucidate the cellular mechanism of neural control of the cellular activity of tracheal smooth muscles, we recorded changes in the membrane potential of the tracheal muscle of dogs by a single or repetitive nerve stimulation using the double sucrose gap or micro-electrode methods.

We found that endogenous prostaglandins appear to play an important physiological role in the negative feed-back mechanism related to excitatory cholinergic transmission.

METHODS

Adult mongrel dogs of either sex, weighing 13–15 kg were anaesthetized with an I.V. administration of pentobarbitone (30 mg/kg). Segments of cervical trachea were excised, and a dorsal strip of transversely running smooth muscle fibre was separated from the cartilage. The mucosa and adventitial areolar tissue were carefully removed. The tracheal smooth muscle was cut at a width of 2·0–2·5 mm and a length of about 20 mm for the double sucrose gap method. The preparation was bathed in a modified Krebs solution with the following ionic concentration (mM), Na^+ 137·4, K^+ 5·9, Mg^{2+} 1·2, Ca^{2+} 2·5, Cl^- 134·0, $H_2PO_4^-$ 1·2, HCO_3^- 15·5 and glucose 11·5. The solution was aerated with 97% O_2 and 3% CO_2 and pH was adjusted to 7·3–7·4 by adding NaOH (0·1 N).

For intracellular recording of the membrane potential from the single cell, thin strips of tissue 15–20 mm in length, 4–5 mm in width and 0·3–0·4 mm thick were used. A conventional micro-electrode filled with 3 M-KCl was inserted from the outer surface of the preparation. Field stimulation (stimulus duration of 50–100 μsec) was applied to the nerve terminals (Kuriyama, 1964). A chamber in which the strips were mounted had a volume of 2 ml., and was superfused at a rate of 3 ml./min at a temperature 35–36 °C.

The double sucrose gap method was also used to record the membrane potential and tension development in the tissue. The chamber used has been described elsewhere (Ito, Suzuki & Kuriyama, 1977). To produce neurogenic responses, field stimulation was applied by a pair of electrodes placed in the centre pool of the apparatus. These electrodes were situated so that a current pulse would pass transversely across the tissue. Single and repetitive stimulations were applied, using a current pulse of 50–100 μsec in duration and about 10–30 V in strength.

To investigate the mechanical properties, the tissue was mounted in a 1 ml. organ bath through which the test solution, at a temperature of 35 °C, flowed continuously (0·2 ml./sec). The preparation was placed vertically and the ends were tied with silk thread. One end of the strip was tied to a mechanotransducer (Nihon-Kohden Ltd. RCA-5743) and the other end to a hook at the bottom of the bath.

The following drugs were used, tetrodotoxin, atropine-sulphate, propranolol-hydrochloride, phentolamine, indomethacin, prostigmine, prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

RESULTS

Analysis of the time course of e.j.p. and passive electrical properties of the tracheal smooth muscle

The mean value of the membrane potential measured with the micro-electrodes was $-59{\cdot}5 \pm 0{\cdot}6$ mV ($n = 150$, $\pm$S.D.), and the membrane was quiescent. In other words, neither spontaneous fluctuation of the membrane potential nor spontaneous action potential was observed. With application of field stimulation (50 μsec in duration), a brief membrane depolarization (excitatory junction potential, e.j.p.) was observed.

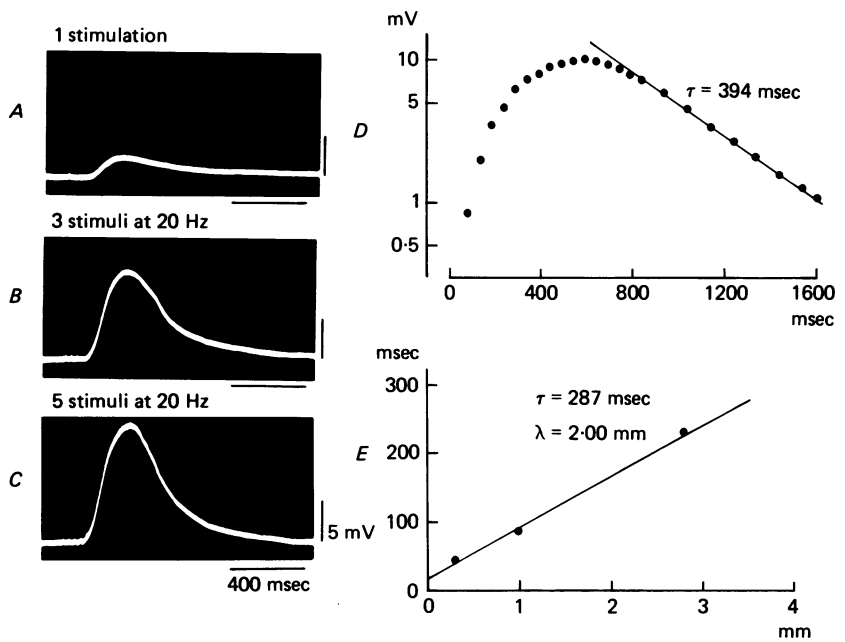

Fig. 1. *A–C*, e.j.p.s recorded by the micro-electrode method, and the number of stimulations were 1, 3 and 5 at the stimulus frequency of 20 Hz, respectively. The distance between the stimulating and recording electrode was 30 μm. *D*, semilogarithmic plot of decay of e.j.p. *E*, linear relationship between the time required to reach a half amplitude of the electrotonic potential induced by the inward current pulse and distance from the stimulating electrode.

The amplitude of e.j.p.s recorded by the micro-electrode was in a wide range between 0·5 and 10 mV and depended mainly on the distance from the stimulating electrode, the stimulus intensity or duration. E.j.p.s could be recorded when the micro-electrode was inserted into the cells within 2 mm from the stimulating electrode. Fig. 1 (*A–C*) shows e.j.p.s recorded from the cell which was about 30 μm from the stimulating electrode. As shown in Fig. 1 *B* and *C*, the amplitude of the e.j.p. increased in proportion to the number of stimuli at the constant stimulus intensity and frequency. Increase in the stimulus intensity also enhanced the amplitude of e.j.p. with no change in the latency. When a single stimulation was applied, the latency between the stimulus artifact and the onset of the e.j.p.s was 118 msec in this

particular cell. The mean latency measured from seventy-one cells was 298·7 ± 112·0 msec (± S.D.).

The time required to reach the peak amplitude of the e.j.p.s was in the range of 380–950 msec, and the mean value was 531·1 ± 120·1 msec (± S.D., $n = 71$). Decay of the e.j.p. evoked by a single stimulation plotted on a semilog scale was linear, and the time constants were in the range of 350–421 msec, the mean value being 393·0 ± 16·0 msec ($n = 10$, ± S.D.).

To measure the time constant of the tissue, square pulses of 2 sec in duration and different intensities were applied to the tissue by the partition stimulating method (Abe & Tomita, 1968), and the amplitudes of electrotonic potential were measured at various distances from the stimulating electrode. The mean value of the length constant (λ) was 2·13 ± 0·26 mm (± S.D., $n = 5$).

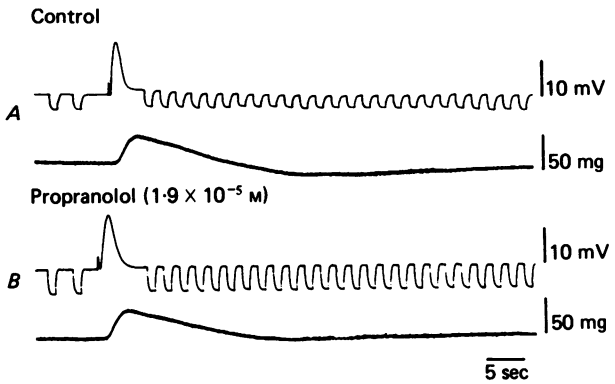

Fig. 2. Effects of field stimulation on the electrical and mechanical properties of dog tracheal muscle observed with the double sucrose gap method. E.j.p. evoked by three stimuli at 20 Hz (50 μsec in duration, and 20 V in intensity) and following twitch and subsequent relaxation. *A*, changes in the amplitude of the electrotonic potentials evoked by the constant inward current pulses (1·5 sec duration) during the prolonged relaxation evoked by the field stimulation. *B*, effects of propranolol on the amplitude of prolonged relaxation or the electrotonic potentials induced by the constant current pulses.

When the time required to reach a half of the final steady amplitude of the electrotonic potential was plotted against the distance from the stimulating electrode, a linear relationship was observed (Fig. 1*E*).

From the cable equation, the slope can be expressed as $\tau/2\lambda$ (Hodgkin & Rushton, 1946), where τ is the time constant of the membrane at erf 1 and λ is the length constant of the membrane. The mean time constant of the tracheal circular muscle was 305·7 ± 37·9 msec ($n = 4$). The decay of e.j.p.s was obviously slower than the passive decay of the electrotonic potentials, presumably due to the distance between the nerve terminals and the effector cells.

Effects of field stimulation on the electrical and mechanical properties of tracheal smooth muscle

Application of field stimulations (50 μsec in duration) by the double sucrose gap method produced e.j.p.s (3–4 sec duration) and such were followed by biphasic changes in the mechanical property, i.e. the initial twitch-type contraction and subsequent long lasting relaxation of the muscle tissue (Fig. 2*A*).

E.j.p.s induced by field stimulation were completely blocked by treatment with either tetrodotoxin (2×10^{-7} M) or atropine ($1{\cdot}7 \times 10^{-5}$ M).

The amplitude of the e.j.p.s evoked during single field stimulation by the double sucrose gap method ranged between 1 and 5 mV, and the responses were graded according to the strength and duration of the stimulation impulses. With field stimulation or direct muscle stimulation, action potential in the tracheal smooth muscle was not produced, and the amplitude of twitch contraction depended on the amplitude of e.j.p.s.

Under treatment with atropine, field stimulation did not evoke e.j.p.s. However, after a delay of several seconds, the tension decreased to a level below the base line, followed by a slow recovery to the control level. The amplitude of the relaxation was enhanced in the presence of atropine by increases in the stimulus intensity or in particular by increases in the number of stimuli at high frequencies (10–30 Hz). The relaxation induced by the field stimulation was completely suppressed by treatment with either tetrodotoxin (2×10^{-7} M) or propranolol ($1{\cdot}9 \times 10^{-5}$ M) (Fig. 2*B*). To observe changes in the membrane potential during the relaxation, the micro-electrode method was used. After the pre-treatment with atropine (2×10^{-5} M), the membrane potential was recorded from the same cell throughout the experiments before and after the application of field stimulation. However, there was no apparent change in the membrane potential (the mean value was $-59{\cdot}0 \pm 0{\cdot}9$ mV, $n = 9$, $\pm$S.D.).

To investigate changes in the input membrane resistance during the relaxation induced by the field stimulation, electrotonic potentials were evoked before, during and after application of the field stimulation. As shown in Fig. 2*A*, the amplitude of electrotonic potentials induced by the constant rectangular pulses (1·5 sec in pulse duration) was gradually reduced after the nerve stimulation, and the maximum reduction in the amplitude of electrotonic potential was observed before the relaxation reached the peak level. The amplitude gradually then returned to the control value. The maximum reduction in the amplitude of electrotonic potential to the control value (before the stimulation) was 21·1 % in this experiment. The mean value of the reduction in the input membrane resistance, as determined from five experiments was $15{\cdot}3 \pm 5{\cdot}6$ % ($n = 5$, $\pm$S.D.). In the presence of propranolol ($1{\cdot}9 \times 10^{-5}$ M), neither reduction in the input resistance nor relaxation occurred (Fig. 2*B*). Thus, the relaxation induced by field stimulation is mainly mediated by activation of β-adrenergic receptors due to activation of adrenergic nerve fibres.

Changes in the amplitude of e.j.p.s during prolonged exposure in Krebs solution

The size of e.j.p.s evoked by field stimulations with a fixed stimulus intensity and duration gradually and continuously decreased over 1–2 hr. In the preparations of vas deferens or ileal longitudinal muscle of guinea-pig there was no such reduction in the amplitude, and evidence of a steady level was apparent within 1 hr after the start of experiments (Ito & Tajima, 1979, 1980).

Field stimulations activated both cholinergic and adrenergic nerve fibres in this preparation. Therefore, one possibility is that catecholamines released during the repetitive field stimulations act on the cholinergic nerve terminals, thus causing a reduction in the amplitude of e.j.p.s. The adrenergic link in the cholinergic transmission occur in different anatomical locations (Krnjević & Miledi, 1958; Hidaka & Kuriyama, 1969; Kuba, 1970; Kuba & Tomita, 1971). However, the amplitude of

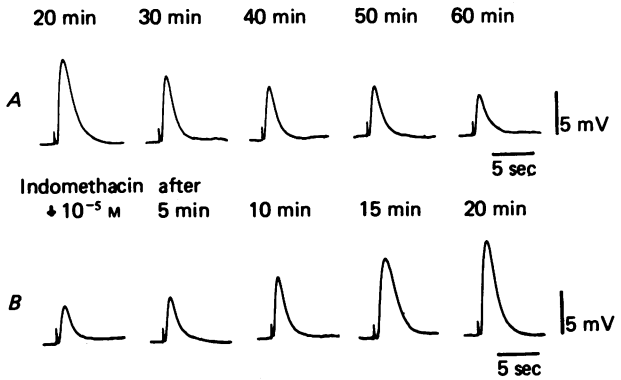

Fig. 3. *A*, changes in the amplitude of e.j.p.s during the time course of series of experiment in the presence of phentolamine (10^{-5} M) and propranolol ($1{\cdot}9 \times 10^{-5}$ M). Single stimulation (50 μsec in duration, 25 V in intensity) was applied every 5 min in the time course of the experiments. *B*, effects of indomethacin (10^{-5} M) on the e.j.p.s elicited by a single field stimulation in the presence of phentolamine (10^{-5} M) and propranolol ($1{\cdot}9 \times 10^{-5}$ M).

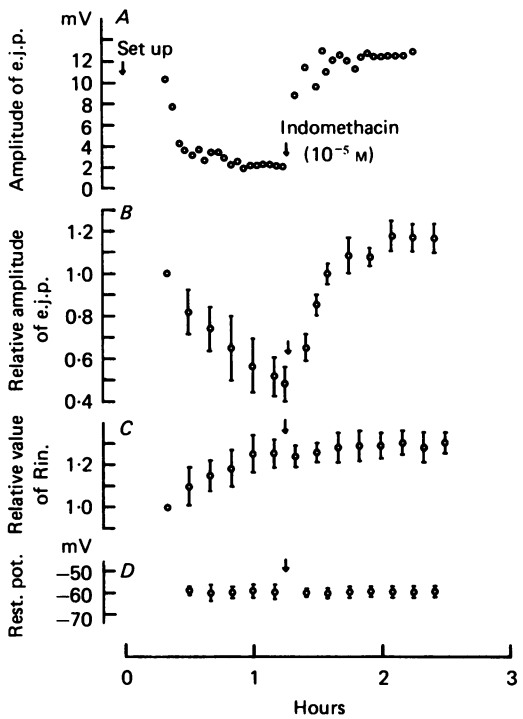

Fig. 4. Changes in the amplitude of e.j.p., resting membrane potential or the input membrane resistance during the time course of experiments. *A* represents changes in the amplitude of e.j.p.s in a series of experiments. *B* shows relative changes in the amplitude of e.j.p. measured from six experiments, where the amplitude of e.j.p. evoked 20 min after the start of experiment was defined as 1·0. *C*, relative changes in the input membrane resistance, the input membrane resistance 20 min after the start of experiment was registered as 1·0. *D*, mean values of resting membrane potential recorded from ten to twenty-five cells during the time course of six experiments. Vertial bars indicate 2 S.D.

e.j.p.s elicited every 5 min by the constant field stimulations was also gradually reduced in the presence of the adrenergic blockers phentolamine (10^{-5} M) and propranolol ($1{\cdot}9 \times 10^{-5}$ M). For example, the amplitude of e.j.p. was 11 mV after 20 min superfusion in the double sucrose gap apparatus, and after 60 min was only 5 mV (Fig. 3*A*).

Fig. 4*C* shows changes in the input membrane resistance measured from the amplitude of electrotonic potentials evoked by constant rectangular pulses (1·5 sec), in the case of the double sucrose gap method. The input membrane resistance gradually increased up to 60 min after superfusion with Krebs solution, but the amplitude of e.j.p.s did not reach a steady level for 60 min. The membrane was slightly hyperpolarized with a longer superfusion, however, the change was not statistically significant (Fig. 4*D*).

These results show that reduction in the size of e.j.p.s during prolonged superfusion is not due to changes either in the input membrane resistance or the resting membrane potential.

Effects of indomethacin and prostaglandins on the amplitude of e.j.p.

Lung tissues of all species so far investigated contain high amounts of prostaglandins (Änggård, 1965; Karim, Hillier & Devlin, 1968), and prostaglandins are released spontaneously from perfused whole lung or lung fragments (Yen, Mathé & Dugan, 1976; Mathé, Yen, Sohn & Hedqvist, 1977). It is also known that prostaglandins are released into the incubation media from tracheal tissue (Hedqvist & Mathé, 1977), and that the prostaglandin E series of compounds relaxes tracheobronchial smooth muscle preparations from a number of species, including man (Main, 1964; Horton & Main, 1965; Sweatman & Collier, 1968).

The effects of indomethacin and prostaglandins on the amplitude of e.j.p.s generated from the dog tracheal smooth muscle were investigated in an attempt to determine the cause of the gradual reduction in the amplitude of e.j.p.s.

As shown in Fig. 3*B*, when indomethacin (10^{-5} M) was applied during the reduction in the amplitude of e.j.p.s, the size of these potentials gradually increased and a steady level was evident within 30–50 min.

Experiments were repeated using nine preparations (five dogs), and in no case was there a reduction in amplitude of e.j.p. after the treatment with indomethacin. The relative amplitude of e.j.p.s evoked 20 min after the start of experiments was defined as 1·0, and mean changes in the relative amplitude of e.j.p.s in the six experiments are shown in Fig. 4*B*. After the application of indomethacin (10^{-5} M), a reduction in the amplitude was prevented and there were no effects on the input membrane resistance or the resting membrane potential (Fig. 4*C* and *D*).

When the amplitude of the e.j.p.s became stabilized after indomethacin treatment, the effects of various concentrations of prostaglandins were observed. In the concentration of 10^{-11} M, PGE_2 or PGE_1 reduced the amplitude of e.j.p.s, recorded by the double sucrose gap method, to about 80% of the control value, and the amplitude of e.j.p.s was reduced to $62{\cdot}2 \pm 8{\cdot}6$% ($\pm$S.D., $n = 6$), $33{\cdot}0 \pm 4{\cdot}7$% ($\pm$S.D., $n = 6$), and 14·6% ($\pm$S.D., $n = 6$) with the application of 10^{-10} 10^{-9} and 10^{-8} M–PGE_2, respectively. At 10^{-7} M, PGE_1 or PGE_2 completely suppressed the generation of e.j.p.s (Fig. 5*A*). $PGF_{2\alpha}$ in the concentration of 10^{-11}–10^{-10} M, however, did not modify the

amplitude of e.j.p.s, and at 10^{-9} M–$PGF_{2\alpha}$ the amplitude was reduced to 84·0±5·2% (±S.D., $n = 5$) of the control value. With a further increase in the concentration of $PGF_{2\alpha}$ to 10^{-8} or 10^{-7} M, the amplitude was reduced to 70·5±2·1 (±S.D., $n = 4$) or 54·1±4·6% (±S.D., $n = 4$) respectively.

The membrane resistance measured from the amplitude of electrotonic potentials induced by square pulses and the resting membrane potential as measured with micro-electrodes were not affected by prostaglandins (Fig. 5*B* and *C*).

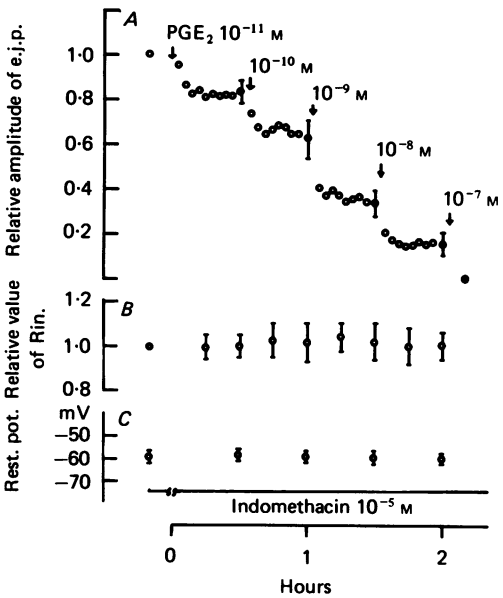

Fig. 5

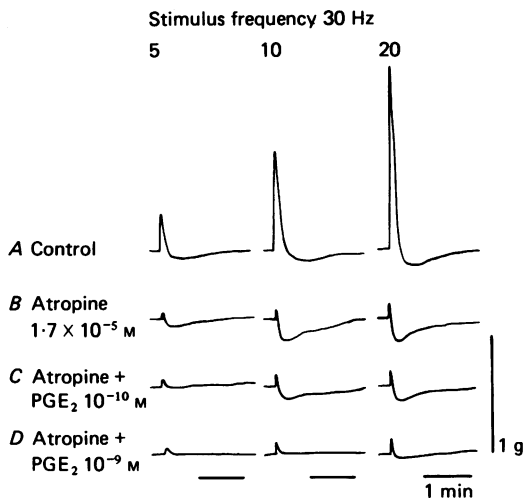

Fig. 6

Fig. 5. Effects of prostaglandin E_2 in various concentrations on the amplitude of e.j.p. (*A*), input membrane resistance (*B*), and the resting membrane potential (*C*). 30 min before the application of PGE_2, the tissues were pre-treated with indomethacin, and the experiments were performed in the presence of indomethacin (10^{-5} M). Vertical bars indicate 2 S.D. (standard deviation). Open and filled circles with vertical bars indicate the mean value calculated from five experiments.

Fig. 6. Mechanical responses induced by the field stimulations and the effects of PGE_2 on the amplitude of relaxation. *A* and *B*, effects of number of stimuli at 30 Hz (500 μsec in duration) in absence and presence of atropine ($1·7 \times 10^{-5}$ M). *C* and *D*, effects of PGE_2 on the amplitude of relaxation in the presence of atropine ($1·7 \times 10^{-5}$ M).

The effects of prostaglandins on the amplitude of relaxation induced by field stimulation were also observed using the tension recording method. Fig. 6*A* shows the mechanical responses to field stimulation with a different number of stimuli. Amplitudes of the twitch contraction and the following relaxation increased in proportion to the number of stimuli (500 μsec pulse duration and 30 Hz). When atropine ($1·7 \times 10^{-5}$ M) was added to the Krebs solution, the twitch contraction was markedly suppressed but the amplitude of relaxation was increased. The relaxation was markedly suppressed in the presence of 10^{-10} M-PGE_2 and was all but abolished with a concentration of 10^{-9} M, under conditions of pre-treatment with $1·7 \times 10^{-5}$ M-atropine.

Effects of repetitive stimulations on the amplitude of the e.j.p.

The effects of repetitive field stimulation (0·1–1 Hz) on the amplitude of e.j.p. and the twitch tension, were observed in the case of pre-treatment with indomethacin (10^{-5} M). Fig. 7*A* and *B* represents e.j.p.s and twitch tensions evoked by repetitive field stimulations at the frequency of 0·1 and 0·2 Hz (pulse duration was 50 μsec). A remarkable depression in the amplitude of e.j.p.s was observed during the repetitive

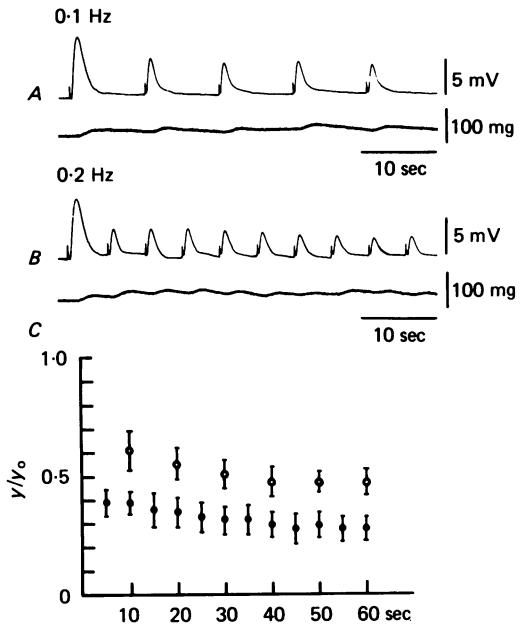

Fig. 7. Effects of repetitive stimulation at the frequency of 0·1 (*A*) or 0·2 Hz (*B*) on the amplitude of e.j.p. and twitch tension of the dog trachea. *C*, relative changes in amplitude of e.j.p.s during the repetitive stimulations measured from 5 experiments. Open and filled circles indicate stimulus frequency of 0·1 and 0·2 Hz respectively. Vertical bars show 2 S.D.

stimulation. The duration of the twitch was much longer than that of the e.j.p.s., and the summations of the contractions were observed at the low stimulus frequency of 0·1 Hz, even though the e.j.p.s showed a depression in the amplitude during the repetitive stimulations. Fig. 7*C* shows the time course of depression in the amplitude of e.j.p.s measured from five experiments, at the stimulus frequency of 0·1 or 0·2 Hz. The relative amplitude of e.j.p. evoked by the first impulse was defined as 1·0.

To analyse the depression in the amplitude of e.j.p.s, changes in the amplitude of a test e.j.p. evoked at different intervals after application of a conditioning impulse were observed in the presence of indomethacin. Fig. 8 (*A–C*) represents one of the series experiment, and Fig. 8*D* shows the mean value of the relative amplitude of the test e.j.p. to the first e.j.p. (1·0) measured from nine experiments.

Depression can be expressed as

$$D = 1 - Y/Y_0,$$

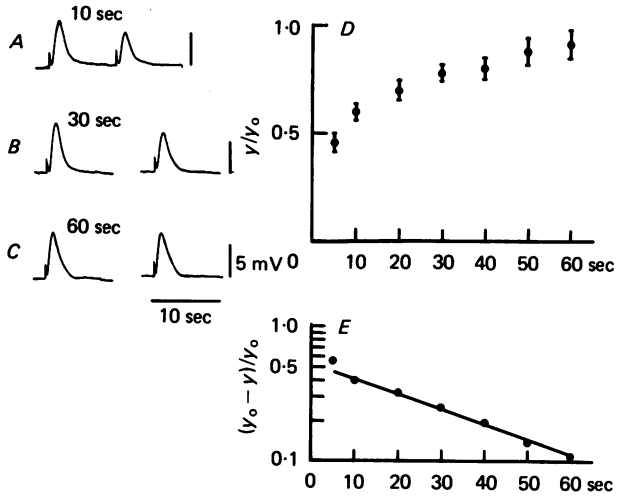

Fig. 8. *A–C*, effects of conditioning e.j.p.s on the amplitude of test e.j.p. evoked at various time intervals in the normal Krebs solution. *D*, mean amplitude of the test e.j.p. after a conditioning e.j.p. measured from five experiments. *E*, the relationship between log $(Y_0 - Y)/Y_0$ and time interval between the two stimuli, where Y_0 is the amplitude of the conditioning e.j.p. (mV) and Y is the amplitude of test e.j.p. Each point gives the mean amplitude of several trials.

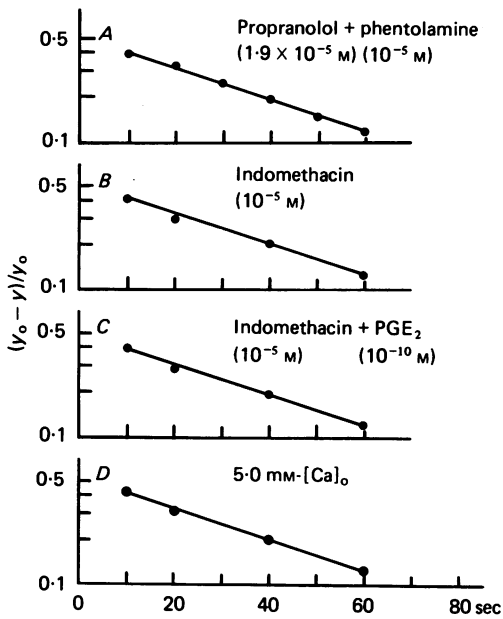

Fig. 9. Effects of various agents on the parameters related to the depression observed with double stimulation experiments.

where Y = amplitude of the test e.j.p.s, and Y_0 = amplitude of the first conditioning e.j.p. When the depression of test e.j.p.s evoked at different intervals after a conditioning e.j.p. was plotted on a log scale against the time interval, the depression could be classified into two components, i.e. the first component occurred with stimulations of short intervals within 10 sec, and the second component with intervals over 10 sec (Fig. 8*E*). The second component of Fig. 8*E* can be expressed by the equation $D = D_0 \exp(-bt)$, where D_0 is the depression at zero time (equal to 0·6) and b is the rate constant of decay of the curve (equal to 0·028 sec^{-1}).

In hopes of providing an explanation for the long lasting depression of the e.j.p. (over 60 sec), the experiments of the double stimulation were repeated in the presence of propranolol ($1{\cdot}9 \times 10^{-5}$ M) with phentolamine (10^{-5} M), indomethacin (10^{-5} M), indomethacin (10^{-5} M) with PGE_2 (10^{-10} M), and 5·0 mM-$[Ca^{2+}]_0$ (Fig. 9). From the slopes of the straight line between log $(1 - Y/Y_0)$ and the time interval, the rate constants of the depression were calculated. The values were not significantly different from the value obtained in the normal Krebs solution.

Increase in the concentration of $[Ca]_0$ from 2·5 to 5·0 mM enhanced the amplitude of e.j.p. to $1{\cdot}61 \pm 0{\cdot}10$ ($\pm$S.D., $n = 3$) times the control values, however the rate constant and the depression at zero time were not affected.

DISCUSSION

With application of single or repetitive field stimulation, e.j.p.s were generated in the dog tracheal smooth muscle and such was followed by a twitch tension and a subsequent long lasting relaxation of the tissue. These electrical and mechanical responses were blocked by tetrodotoxin. Atropine suppressed both the e.j.p.s and twitch tension, and propranolol blocked the generation of the long lasting relaxation. This means that the dog tracheal smooth muscles are innervated by cholinergic excitatory and adrenergic inhibitory systems, but not by non-adrenergic non-cholinergic inhibitory systems (Suzuki, Morita & Kuriyama, 1976).

The existence of non-adrenergic, non-cholinergic inhibition in the trachea of the guinea-pig has been demonstrated by Coburn & Tomita (1973) and subsequent authors (Coleman & Levy, 1974). In humans, tracheal and bronchial smooth muscle is controlled principally through cholinergic excitatory nerve fibres and non-adrenergic, non-cholinergic inhibitory nerves with no direct inhibition of the smooth muscle by adrenergic fibres (Richardson & Beland, 1976). Thus, there are marked differences in the innervations in the tracheal muscles of dog, guinea-pig and humans.

E.j.p.s recorded from the dog tracheal smooth muscle by the micro-electrode were of a long duration and latency as measured from the interval between the stimulus artifact and the onset of e.j.p.s. Cameron & Kirkpatrick (1977) using the double sucrose gap method also reported findings of a long latency and prolonged duration of e.j.p.s in tissues from the bovine trachea and concluded that the latency was partly due to the conduction time down to the fine nerve terminals from the stimulus site to the interface between Krebs solution and sucrose (a distance of 3 mm). In the present experiments, the minimum latency required to generate the e.j.p. was 118 msec from the cell distributed within 30 μm from the stimulating electrode.

Therefore, the long latency for the generation of e.j.p.s is probably not due to slow conduction time from the stimulating electrode to the recording sites.

Since the innervation of the muscle tissues is reportedly sparse in the case of bovine or dog trachea (Suzuki *et al.* 1976; Cameron & Kirkpatrick, 1977), diffuse liberations of transmitters may partly explain the long latency and the slow rising and falling phases of the e.j.p. Furthermore, it is known that a long latency is one of the characteristics of muscarinic responses in many tissues (Purves, 1976).

E.j.p.s recorded from the dog trachea showed a depression during repetitive field stimulation. Double stimulus experiments at different intervals revealed that the depression lasts for over 1 min, and could be classified into two components, i.e. the first component occurred with stimulations of short intervals within 10 sec, and the second component at intervals of over 10 sec. Field stimulation had an excitation effect on both of cholinergic and adrenergic nerve fibres, and recent investigations showed that endogenously released noradrenaline can partially inhibit bronchoconstrictions caused by stimulation of the cholinergic nerves (Vermeire & Vanhoutte, 1979). In the presence of α- and β-adrenergic blocking agents, however, the rate constant for the depression or the depression at zero time did not differ from the values observed in the normal Krebs solution. Furthermore, the application of indomethacin or PGE_2, did not affect the rate constant of the depression, although PGE_2 or indomethacin markedly suppressed or enhanced the amplitude of e.j.p.s. The applications of prostaglandins induced a reduction in the amplitude of the e.j.p.s, but did not affect the depression observed in the case of double stimulation experiments. Therefore, the depression seen during the repetitive nerve stimulation seems to be due to the characteristics of the nerve terminal itself. A similar depression was seen in bovine tracheal tissues (Cameron & Kirkpatrick, 1977).

A most striking feature of the e.j.p.s recorded from the dog trachea is the gradual and consistent reduction in the amplitude during the prolonged superfusion with normal Krebs solution, although a stable membrane potential or input membrane resistance was obtained. With the application of indomethacin (10^{-5} M), however, initial increase in the amplitude of e.j.p.s was observed, and in the presence of indomethacin, e.j.p.s of constant amplitude were obtained for 1–1·5 hr. Furthermore application of PGE_1 or PGE_2 after the pre-treatment with indomethacin decreased the amplitude of e.j.p. with a low concentration of 10^{-11} M, and an increase in the concentration of the PGE series (10^{-7} M) completely suppressed the generation of e.j.p.s without changing either the resting membrane potential or the input membrane resistance. $PGF_{2\alpha}$ also reduced the amplitude of e.j.p., however, higher doses were required than in the case of other two compounds (more than 100 times). Thus, it seems probable that indomethacin enhances the amplitude of e.j.p. through an inhibitory action on the prostaglandin biosynthesis (see for example Flower, 1974; Vane, 1976) rather than by a direct action on the e.j.p., and the nerve terminals of the vagus in the dog trachea possess a specific prostaglandin receptor as in the case of sympathetic nerve terminals in the guinea pig vas deferens (Ito & Tajima, 1979). Prostaglandins (10^{-10}–10^{-9} M) also reduced the amplitude of relaxation induced by the field stimulations without affecting the resting tension of the tissue. Thus, the possibility that nerve terminals of the adrenergic fibres may also possess specific prostaglandin receptors has to be considered.

Prostaglandins affect the tone of airway smooth muscles. Briefly, the prostaglandin E series relaxes tracheobronchial smooth muscle in several species including humans, and $PGF_{2\alpha}$ is a potent and consistent bronchoconstricting agent (Main, 1964; Horton & Main, 1965; Sweatman & Collier, 1968; Mathé, Strandberg & Åström, 1971). The constriction elicited by $PGF_{2\alpha}$ *in vitro* is not affected by atropine, mepyramine, methysergide, α- and β-adrenergic blocking agents and is therefore considered to be a direct action on the smooth muscle cells, possibly through a specific prostaglandin receptor (Mathé, 1976; Smith, 1976; Änggård & Bergström, 1963). However, the concentrations of $PGF_{2\alpha}$ or compounds of the PGE series, in which these agents contract or relax the bronchial smooth muscle, were in the range between 10^{-6} and 10^{-4} M. Thus, the nerve terminals of the cholinergic or the adrenergic nerve fibres are far more sensitive to prostaglandins than are the smooth muscle cells in the trachea. In the presence of hexamethonium (10^{-6} M), prostaglandins also suppressed the amplitude of the e.j.p. in the dog trachea (Y. Ito, unpublished observations). Therefore, if endogenous prostaglandins do play a physiological role in the regulation of the motility of the tracheal muscle, the action would necessarily be mediated through the prostaglandin receptors in the vagal nerve terminals which innervate the smooth muscle cells. Cholinergic rather than adrenergic innervations are dominant in the dog trachea (Suzuki *et al.* 1976). In addition, it has been reported that the amount of PGE or $PGF_{2\alpha}$ released into the incubation medium was 1·9 or 1·7 ng/g wet wt. tissue.min (Hedqvist & Mathé, 1977).

Thus, the presence and release of prostaglandins in the tracheal muscle tissues, the direct actions of prostaglandins in low concentration, or the actions of indomethacin on the amplitude of e.j.p., strongly indicate that prostaglandins play a physiological role in endogenous feed-back inhibitory mechanisms related to acetylcholine release by nerve stimulation in the dog tracheal smooth muscle.

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