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SPONTANEOUS ACTIVITY IN THE TRACHEA OF DOGS TREATED WITH INDOMETHACIN: AN EXPERIMENTAL MODEL FOR ASPIRIN-RELATED ASTHMA

YUSHI ITO & KIKUO TAJIMA

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

1 Electrical and mechanical properties of smooth muscle cells or of neuro-effector transmission in the smooth muscle layer of the dog trachea, were studied after treatment with indomethacin, by means of the double sucrose gap, microelectrode or tension recording methods.

2 After several subcutaneous injections of indomethacin (1.0 mg/kg daily), 6 out of 12 dogs were coughing and wheezing.

3 Smooth muscle tissues dissected from the trachea of the coughing dog showed spontaneous electrical and mechanical activities at the frequency of 8–15 per min. These spontaneous electrical and mechanical activities were completely suppressed by treatment with atropine (10^{-6} M), isoprenaline (5×10^{-7} M) or prostaglandin E_2 (10^{-9} M) but not by tetrodotoxin (1.5×10^{-6} M).

4 Direct muscle stimulation induced oscillatory potential changes followed by tension development in the trachea of the indomethacin-treated dog.

5 In the indomethacin-treated dog, mean membrane potential of the tracheal smooth muscle cells was −52.4 mV, and in the control trachea, the potential was −59.0 mV.

6 In the trachea from control dogs, the amplitude of test e.j.ps after conditioning e.j.ps was always smaller than the conditioning e.j.p., at any time interval between the two stimuli. In the trachea from indomethacin-treated dogs, facilitation phenomena were observed.

7 In the trachea from the indomethacin-treated dog, prostaglandin E_1 (PGE_1) or PGE_2 (10^{-10}–10^{-9} M) markedly suppressed the amplitude of the e.j.p. but did not affect the facilitation phenomenon.

8 These results indicate that endogenous prostaglandins play important physiological roles in the feed-back inhibitory mechanisms for acetylcholine release from the nerve terminals during the resting and active states.

9 The results are also discussed in relation to the genesis of aspirin-induced asthma in man.

Introduction

It has been reported that in some patients with bronchial asthma, aspirin induces acute asthmatic attacks (Samter & Beers, 1967). It has also been reported that ingestion of indomethacin is associated with an acute asthmatic attack in some patients with intrinsic bronchial asthma (Vanselow & Smith, 1967). In previous work (Ito & Tajima, 1981), we found that continuous reduction in the amplitude of excitatory junction potentials (e.j.ps) recorded from dog trachea could be overcome by pretreatment of the preparation with indomethacin, and that the e.j.ps of constant amplitude in the presence of indomethacin were decreased by exogenous application of the prostaglandin E series, in low concentrations.

If endogenously synthesized prostaglandins actually do play an essential role in the feedback inhibitory mechanisms for acetylcholine release from nerve terminals in the tracheal muscle tissue, prolonged treatment with indomethacin might affect the excitatory neuro-effector transmission or the motility of the dog tracheal smooth muscle.

We have studied the electrical and mechanical properties of the smooth muscle cells and neuro-effector transmission in the dog trachea following administration of indomethacin. The results are discussed in relation to aspirin-induced asthma observed in patients.

Methods

Indomethacin (Sigma Ltd.) was suspended in peanut oil (Ishizu Pharmac. Ltd.). Twelve, adult mongrel dogs, of either sex, weighing 14–15 kg were given indomethacin subcutaneously in doses of 1.0 mg/kg body weight per day for 5 days. The control dogs were given injections of an equal volume of the vehicle only. The dogs were anaesthetized with pentobar-

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bitone (30 mg/kg) intravenously, and circular muscle strips were prepared from the cervical trachea.

A modified Krebs solution (hereafter referred to as Krebs) of the following composition was used (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, HCO_3^- 15.5 $H_2PO_4^-$ 1.2, and glucose 11.5 equilibrated with 97% O_2 and 3% CO_2.

Apparatus and experimental procedures for the microelectrode, the double sucrose gap or tension recording methods were the same as those described by Ito & Tajima (1981). The following drugs were used: indomethacin, tetrodotoxin, atropine sulphate, (−)-isoprenaline hydrochloride and prostaglandin E_2.

Results

Mechanical properties of the tracheal muscle

After three or four injections of indomethacin, 6 out of 12 dogs began to wheeze or cough about 10–20 times per min. This did not happen in the control dogs.

To assess the nature of this induced wheezing and coughing, we examined the response of the tracheal smooth muscle using the tension recording or double sucrose gap methods.

Figure 1 shows spontaneous contractions of tracheal smooth muscle excised from the dogs with induced spontaneous coughing. The frequency of contractions was in the range of 8 to 15 per min, the mean value being 12.2 ± 2.1 min (n=7, ± s.d.). Tracheal muscles excised from the coughing dogs showed spontaneous mechanical activity. Rapid phasic spontaneous contractions and relaxations appeared intermittently, and at 2 to 5 min intervals, relatively large relaxations occurred.

In the control dog trachea, these spontaneous contractions were not observed, and similar observations have been made by other workers (Kirkpatrick, 1975; Suzuki, Morita & Kuriyama, 1976; Cameron & Kirkpatrick, 1977).

To investigate the nature of the spontaneous contractions, mechanical activity was recorded in the presence of various chemical agents. Tetrodotoxin (1.5×10^{-6} M) had no effect on the amplitude or the frequency of the spontaneous mechanical activity, while atropine (10^{-6} M), isoprenaline (5×10^{-7} M) or prostaglandin E_2 (PGE_2) (10^{-9} M) gradually reduced the amplitude of the phasic contraction and completely suppressed its generation. Isoprenaline also greatly reduced the resting tension (Figure 1).

Electrical and mechanical properties of the trachea treated with indomethacin

The mean value of the membrane potential measured by the microelectrode method was −52.4 ± 1.8 mV (n=55, ± s.d.) in the trachea of the indomethacin-treated dog, and with application of atropine (5×10^{-6} M), the membrane was hyperpolarized to −59.6 ± 1.1 mV (n=50, ± s.d.). The mean value measured in the control dog trachea was −59.0 ± 2.1 mV (n=55, ± s.d.).

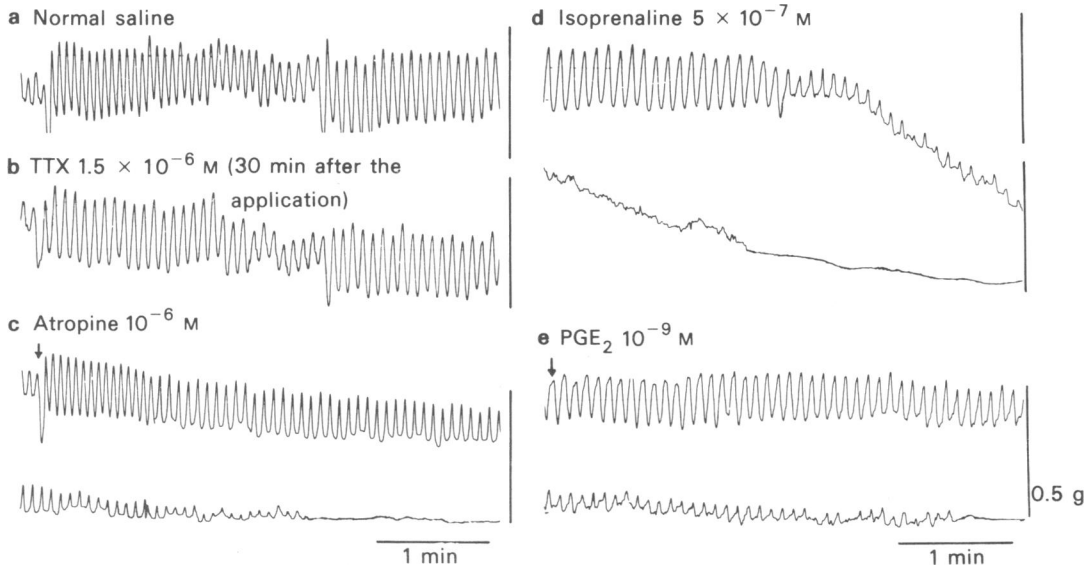

Figure 1 Effects of tetrodotoxin (TTX, 1.5×10^{-6} M), atropine (10^{-6} M), isoprenaline (5×10^{-7} M), or prostaglandin E_2 (PGE_2, 10^{-9} M) on the spontaneous mechanical activity in the trachea of a dog treated with indomethacin for 5 days.

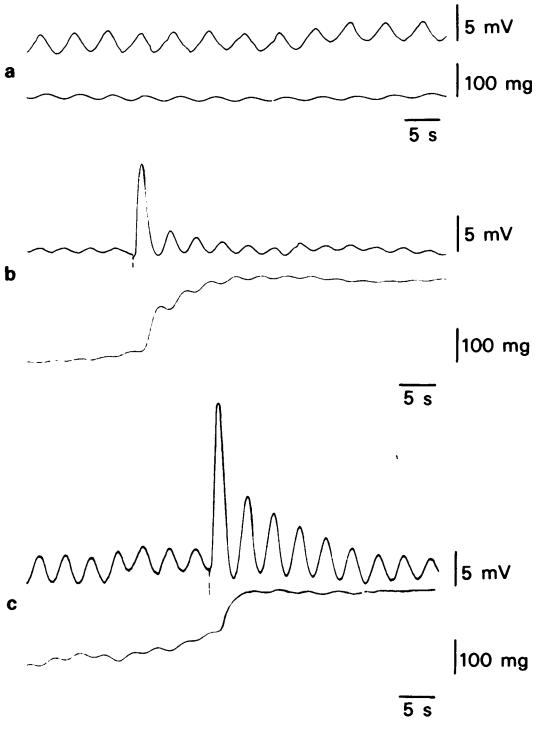

Figure 2 Typical records of oscillatory membrane depolarizations and mechanical activity recorded from tracheal smooth muscles of dog, treated with indomethacin (b and c). Effects of a single field stimulation on the electrical and mechanical activities recorded from a different dog trachea after treatment with indomethacin.

To analyse the spontaneous contractions, changes in the electrical and mechanical properties of the membrane were recorded simultaneously, by the double sucrose gap method. With the double sucrose gap apparatus, slow potential changes followed by contractions were recorded (Figure 2a). The slow depolarization had a duration of 3 to 6 s (mean; 4.3 ± 0.7 s, ± s.d., $n=7$), an amplitude of 1 to 5 mV (mean 3.8 ± 1.1 mV, $n=8$) and a frequency of 6 to 18 per min at 35°C.

The generation of slow depolarizations was blocked by application of atropine (10^{-6} M); however, tetrodotoxin (1.5×10^{-6} M) had no effect on the amplitude or the frequency of the slow potential changes.

With application of field stimulation (50 μs pulse duration), e.j.ps were produced with twitch tension development superimposed on the spontaneous slow contractions. Following the generation of e.j.ps, oscillatory potential changes with a duration of 3 to 5 s and an amplitude of 3 to 10 mV were recorded. These potential changes also generated oscillations in the tension development, i.e. after a single field stimulation, fusion of twitch tensions evoked by e.j.ps and oscillatory contractions occurred, thus producing a tetanus which lasted for several minutes (Figure 2 b, c).

Figure 3 shows changes in the membrane potential and tension induced by the application of outward currents (15 s in pulse duration). In the tracheal smooth muscle excised from the control dogs, the application of outward currents elicited no active response in the membrane (Figure 3 e, f). However, when the prolonged membrane depolarization induced by the outward current (15 s in duration) exceeded 10 mV, the tension developed slowly and with a much smaller amplitude as compared to the twitch tension induced by the e.j.p. with an amplitude of about 10 mV (Figure 3d).

In contrast, in the tracheal tissues from the indomethacin-treated dogs in which there was spontaneous or no mechanical activity, outward current pulses (15 s in pulse duration) induced oscillatory potential changes followed by a rapid increase in the tension development (Figure 3 a, b). The amplitude of the oscillatory potential changes was enhanced with increase in the intensity of the outward current pulses, and oscillatory potential changes were observed after cessation of the current application. These graded and oscillatory potential changes, followed by a rapid and large increase in the tension development were blocked by treatment with atropine (10^{-6} M) but not by tetrodotoxin (1.5×10^{-6} M) (Figure 3 b, c.).

Properties of neuro-effector transmission in the trachea from indomethacin-treated dogs

To compare excitatory neuro-effector transmission in tracheal tissue from control and indomethacin-treated dogs, e.j.ps were recorded with micro-electrodes and the double sucrose gap method. The amplitudes of e.j.ps recorded by the double sucrose gap method from the tracheal tissues of indomethacin-treated dogs were in the wide range of 3 mV to 20 mV, in response to single stimulation (Figure 2 b, c). Direct comparisons of the amplitude were not technically feasible but the mean amplitudes were always larger in the tissues from the indomethacin-treated dogs, in response to the constant stimulus conditions (50 μs in duration, 10–30 V in intensity). Similarly, the amplitude of e.j.ps recorded with micro-electrodes varied from one cell to another; however, e.j.ps with a large amplitude (more than 5 mV) were readily generated in the tracheal tissues from the indomethacin-treated dogs.

Double stimulations of various intervals were applied to compare the amplitude of a conditioning e.j.p. with the amplitude of a test e.j.p. in the control and indomethacin-treated dogs. Figure 4 (a) and (b) shows the actual records obtained from the controls in cases when single or multiple stimuli at 20 Hz were

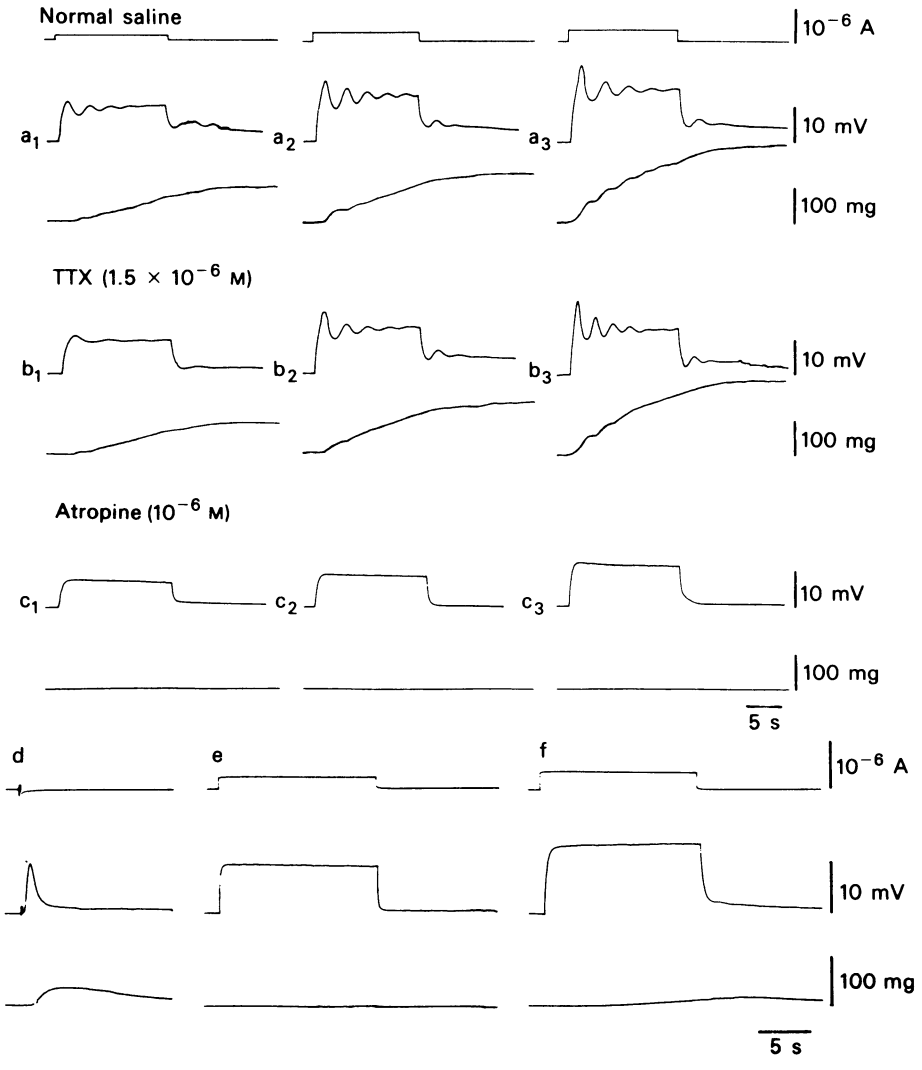

Figure 3 Effects of the direct stimulation of the smooth muscle cells on the electrical and mechanical properties of the trachea, after *in vivo* treatment with indomethacin (a–c). The current intensities were increased from 1 to 3 in a step wise manner. (a) Control; (b) in the presence of tetrodotoxin (TTX, 1.5×10^{-6} M); (c) in the presence of atropine (10^{-6} M); (d) e.j.p. and following twitch tension elicited by field stimulation (5 stimuli at 20 Hz) in the control dog given the vehicle only; (e and f) direct muscle stimulation in the control dog trachea.

used to evoke e.j.ps. The amplitude of test e.j.ps after a conditioning impulse were always smaller than those of conditioning e.j.ps at any time interval, as has been found for non-treated dogs (Ito & Tajima, 1981).

After indomethacin treatment (Figure 4 c, d) the amplitudes of the test e.j.ps were increased. When short intervals (less than 30 s) or multiple stimulations (3 stimuli at 20 Hz) were used to evoke an e.j.p., increase in the amplitude of the test e.j.ps was evident.

Figure 5 (a) and (b) shows relative changes in the amplitude of the test e.j.ps evoked at different time intervals after the application of conditioning impulses. The amplitude of a conditioning e.j.p. was registered as a relative amplitude of 1.0. After indomethacin treatment, the main feature observed during the double stimulations was the facilitation phenomena. However, the amplitude of this facilitation was too small for quantitative analysis.

To elucidate differences in the nature of the tracheal muscle cells from control and indomethacin-treated dogs, repetitive stimulations at high fre-

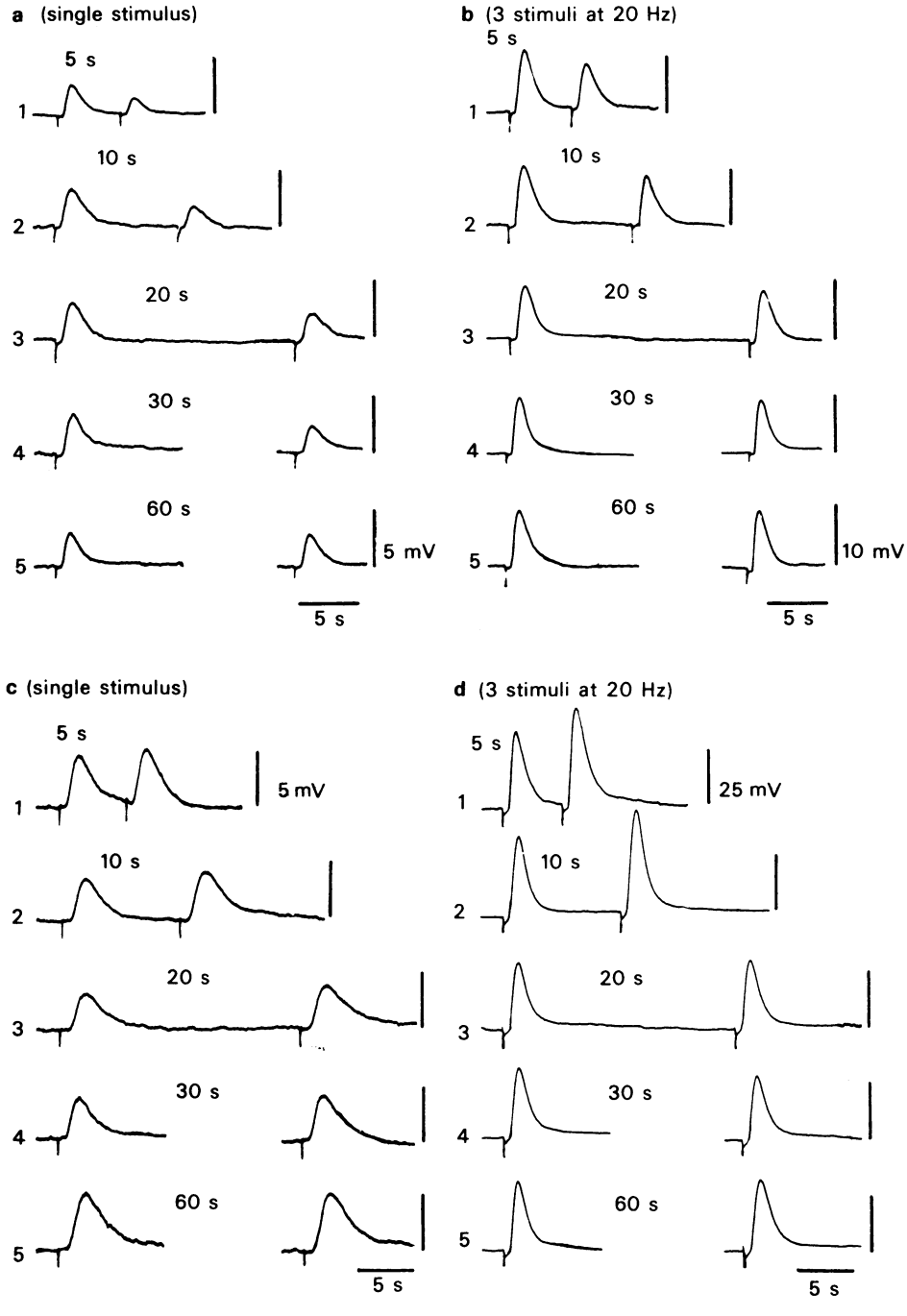

Figure 4 Effects of conditioning e.j.p. on the amplitude of test e.j.p. in the control (a and b) or indomethacin-treated dog trachea (c and d). Single or 3 stimuli at 20 Hz were applied to evoke e.j.ps.

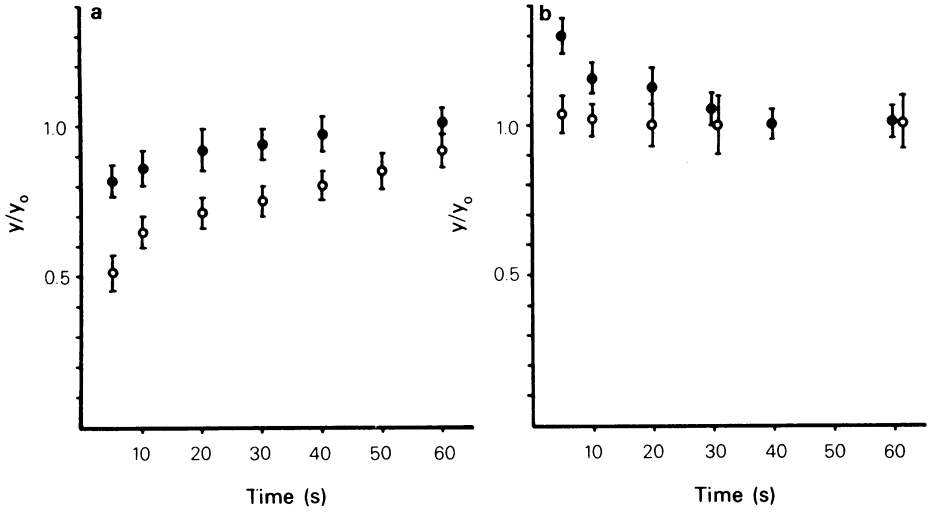

Figure 5 The relative changes in the amplitude of test e.j.ps after the conditioning e.j.ps at various time intervals in the control (a) and indomethacin-treated dog trachea (b). Each point shows the mean value of several experiments, and the vertical bars indicate 2 × s.d. (○) Single stimulus; (●) 3 stimuli at 20 Hz.

quency were applied to observe the change in the amplitude of e.j.ps. Figure 6 shows the relationship between the number of stimuli at 20 Hz and the amplitude of e.j.ps. Here the amplitude of the e.j.p. evoked by a single stimulation was defined as 1.0. When several stimuli at 20 Hz were applied, a linear relationship between the number of stimuli and the amplitude of e.j.p. was observed, and slopes of the straight line were 1.8 and 3.1 in the control and indomethacin-treated dogs, respectively. Thus, the facilitation phenomenon was enhanced in tracheal tissues of the dogs treated with indomethacin.

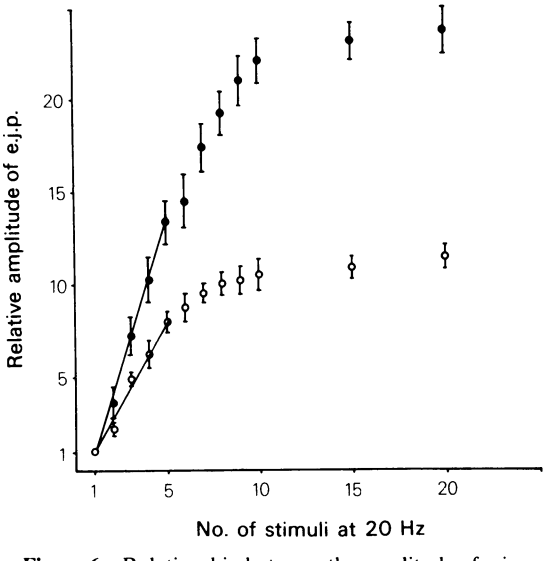

Figure 6 Relationship between the amplitude of e.j.p. and the number of stimuli at 20 Hz. The straight line was fitted by the method of least squares. (○) Control; (●) indomethacin-treated dog trachea. Each point is the mean value of several experiments, and the vertical bars indicate 2 × s.d.

Effects of prostaglandin E series on the amplitude or the facilitation phenomena of e.j.ps in tracheal tissues from dogs treated with indomethacin

In the control dog trachea, the PGE series markedly suppressed the amplitude of e.j.ps. We therefore studied the effects of prostaglandins on the amplitude and the facilitation phenomena of e.j.ps observed in the indomethacin-treated animals.

PGE_1 or PGE_2 markedly suppressed the amplitude of e.j.ps. In low concentrations (10^{-10}–10^{-8} M), the effects of PGE_2 on the amplitude of e.j.ps or the facilitation phenomena were observed in double stimulation experiments (Figure 7 a, b). PGE_2 (10^{-9} M) reduced the amplitude of e.j.ps to 30.1 ± 0.9% (± s.d., n=4) of the control value in trachea from indomethacin-treated dogs and this reduction in the amplitude of e.j.p. was in the same range observed in the controls (Ito & Tajima, 1981). However, in the presence of PGE_2 (10^{-9} M), an increase in the amplitude of the test e.j.p. after a conditioning stimulus was observed at a time interval ranging between 5 to 30 s. The mean increase in the test e.j.ps measured from 4 experiments is shown in Figure 7 c. The increase in the amplitude of the test e.j.p. was in the same range as observed in the absence of PGE_2.

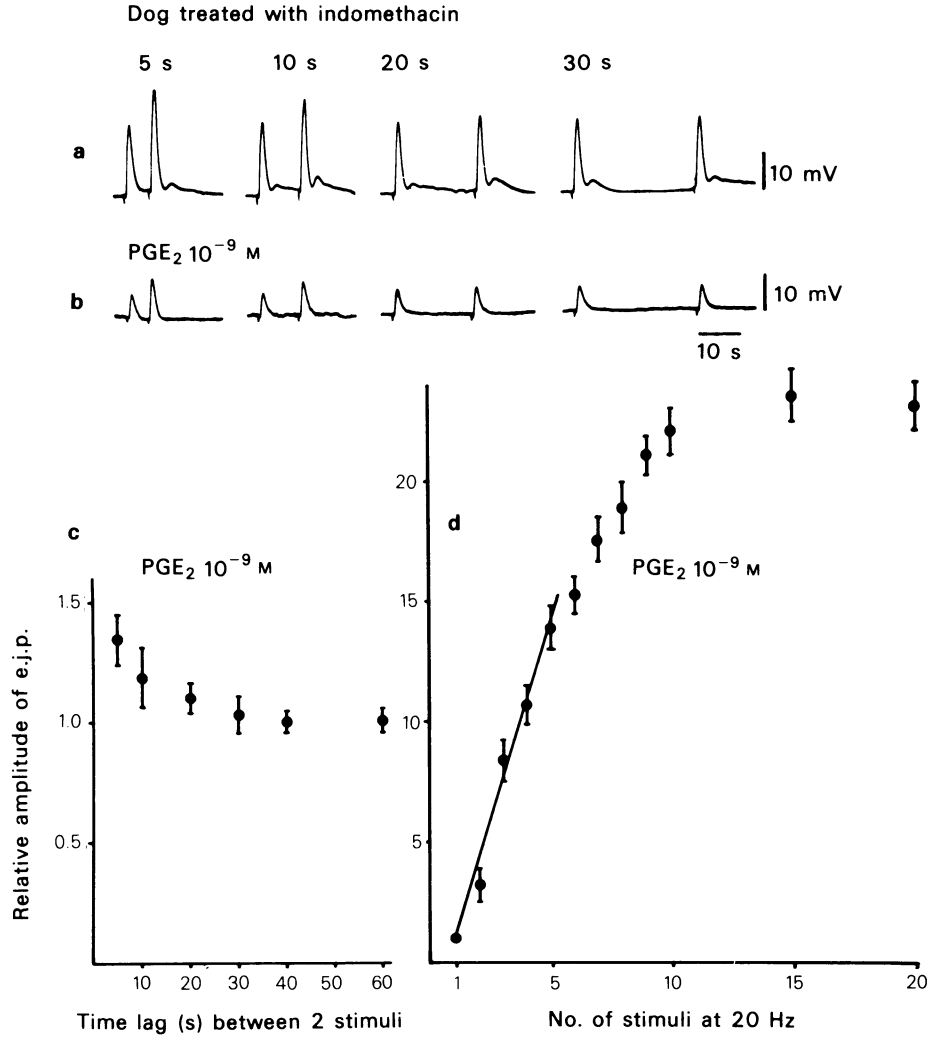

Figure 7 Effects of prostaglandin E_2 (PGE_2) on the amplitude and facilitation phenomena observed in the trachea of the indomethacin-treated dog: (a) in Krebs solution; (b) in the presence of PGE_2 (10^{-9} M); (c) relative changes in the amplitude of test e.j.p. after conditioning e.j.ps; (d) the relationship between the amplitude of e.j.p. and the number of stimuli at 20 Hz.

Figure 7 d shows the relationship between the amplitude of e.j.ps and the number of stimuli at 20 Hz in the presence of PGE_2 (10^{-9} M) where the amplitude of e.j.p. evoked by a single stimulus was defined as 1.0. The linear relation between the amplitude of the e.j.p. and the number of stimuli was observed with application of several stimulations and the slope obtained from the above relationship was 3.2, this value being not significantly different from the value observed in the absence of PGE_2.

These experimental data indicate that PGE_2 suppresses the amplitude of the e.j.p. of the tracheal tissues excised from the indomethacin-treated dogs but does not affect the facilitation phenomenon.

Discussion

Injections of indomethacin (1 mg/kg daily) induced wheezing or coughing in some dogs and the excised tracheal smooth muscle exhibited spontaneous oscillations in the membrane potential with oscillatory contractions.

Similar oscillations in the membrane potential were observed in response to histamine (Kirkpatrick,

1975) or acetylcholine (Cameron & Kirkpatrick, 1977) and to 5-hydroxytryptamine (Coburn & Yamaguchi, 1975) in bovine or dog trachea. In the guinea-pig ileal smooth muscle, acetylcholine or carbachol produce both potential dependent slow waves and spikes (Bolton, 1971; 1973 a,b). These findings in the ileal or tracheal smooth muscle indicate that agents which depolarize the membrane to within a particular range could initiate such oscillations in the membrane potential. In the bovine trachea, oscillatory potentials were observed when the membrane depolarization induced by acetylcholine had reached a level of 12 mV, as measured by the sucrose-gap method (Cameron & Kirkpatrick, 1977).

As the membrane was depolarized (about 10 mV), and oscillatory potential changes with contractions were suppressed by treatment with atropine, it is likely that the spontaneous oscillatory potentials and contractions observed in the tracheal tissue from dogs given indomethacin are induced by acetylcholine released from nerves in the muscular layer.

Depolarization of the membrane by the application of outward currents induced slow oscillatory potentials in the trachea with or without spontaneous activity, and active potential changes were blocked by atropine. However, such oscillatory potentials produced by the application of outward currents did not occur in tissue from the control or untreated dogs. If acetylcholine does induce such oscillatory potential changes, membrane depolarizations caused by acetylcholine may not have been sufficiently extensive to induce spontaneous oscillations in six of the twelve mongrel dogs treated with indomethacin, and oscillations were only evoked with additional membrane depolarization produced by application of outward currents. The spontaneous release of acetylcholine is probably not induced by spontaneous nerve activity, because tetrodotoxin did not affect the generation of spontaneous electrical and mechanical activities. Spontaneous leakage of acetylcholine has been observed in the neuromuscular junction of frog skeletal muscle (Katz & Miledi, 1977) and in the ileal muscle strips with myenteric plexus (Paton & Zar, 1968), and the smooth muscle, epithelium and vagal innervations in the airways have a common embryonic origin with these elements in the gastrointestinal tract (Krahl, 1964).

With indomethacin treatment, the amplitude of e.j.ps, evoked by a single field stimulation or the facilitation phenomenon observed during repetitive stimulation at high frequency (20 Hz) was enhanced in comparison with findings in control dogs. The main feature observed during the double stimulations was the facilitation, and in the control or untreated dogs only the depression was observed.

Application of PGE_2 to the tracheal tissues from dogs treated with indomethacin, reduced the amplitude of e.j.ps, as was also observed in the untreated dogs but did not affect the facilitations observed during repetitive nerve stimulation. The precise mechanisms involved in the reversal actions which change the amplitude from depression to facilitation of e.j.ps during repetitive stimulation are unknown. However, in the cat uterus, the hormonal state was found to be an important factor in determining the type of response produced by the catecholamines (Miller, 1967; Marshall, 1967), therefore prostaglandins seem to play an important hormonal role in regulating the mechanisms involved in transmitter release.

Soon after the finding of aspirin-sensitivity in asthmatic patients (Samter & Beers, 1967) it was reported that in addition to aspirin, other anti-inflammatory drugs were also associated with an acute asthmatic attack in a patient with intrinsic bronchial asthma. These drugs include indomethacin, aminopyrine, tartrazine (a yellow dye frequently present as a food additive), acetoaminophen and mefenamic acid (Vanselow & Smith, 1967; Smith, 1971; Szczeklik, Gryglewski & Czerniawska-Mysik, 1975). Most of these agents have been repeatedly shown to inhibit the cyclo-oxygenase pathway of arachidonate metabolism and interfere with the biosynthesis of PGF, PGE, prostaglandin endoperoxide (PGG_2 and PGH_2) and thromboxane A_2 (see for example, Flower, 1974; Vane, 1976).

The possible roles of prostaglandins in cases of asthma in man centred on the direct action of $PGF_{2\alpha}$ and PGE_2 on the tracheobronchial smooth muscle, because these agents contract or relax bronchial muscle, respectively (Sweatman & Collier, 1968; Cuthbert, 1969; Mathe, Hedqvist, Holmgren & Svanborg, 1973; Parker & Snider, 1973; Dawson & Sweatman, 1975). Prostaglandins can be readily demonstrated in human lungs and lungs of laboratory animals and released in physiologically significant quantities from passively sensitized human lung fractions during challenge with antigens (Piper & Walker, 1973). All these data taken together indicate that the endogenous prostaglandin E series mediate feed-back inhibitory mechanisms for acetylcholine release by nerve stimulation, that the prolonged treatment with idomethacin induces spontaneous electrical and mechanical activities of the trachea, and that the alterations in properties of the nerve terminals result in release of the transmitter.

The dog tracheal muscles are innervated with cholinergic excitatory and adrenergic inhibitory nerves, but not non-cholinergic, non-adrenergic inhibitory nerves (Suzuki *et al.*, 1976). The endogenous feed-back inhibitory mechanisms mediated by prostaglandins for acetylcholine release from the nerve terminals of the vagus may therefore play important physiological roles in the regulation of the motility of the tracheal muscle. Prolonged treatment

with indomethacin removes the endogenous PGEs, and spontaneous contractions of trachea are readily induced. These responses of the dog trachea are representative of one of the features of the aspirin-related asthma observed clinically.

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