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Pre- and post-junctional actions of prostaglandin I_2, carbocyclic thromboxane A_2 and leukotriene C_4 in dog tracheal tissue

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1 Effects of carbocyclic thromboxane A_2 ($cTxA_2$), prostacyclin (PGI_2) or leukotriene C_4 (LTC_4) on the membrane and contractile properties of the smooth muscle cells and on the excitatory neuro-effector transmission in the dog trachea were observed by means of the microelectrode, double sucrose gap and tension recording methods.

2 $cTxA_2$, PGI_2 or LTC_4 at a concentration of 10^{-7}M had no effect on the membrane potential of smooth muscle cells of the dog trachea. At 10^{-6}M, $cTxA_2$ and LTC_4 slightly depolarized, and PGI_2 hyperpolarized the membrane.

3 $cTxA_2$ ($> 2.7 \times 10^{-10}$M) evoked a sustained contraction, while the amplitude of the twitch contractions evoked by field stimulation in the presence of indomethacin (10^{-6}M) and propranolol (10^{-6}M) was inhibited, dose-dependently. PGI_2 ($> 2.7 \times 10^{-7}$M) reduced the muscle tone and the amplitude of twitch contractions evoked by field stimulations.

4 $cTxA_2$ or PGI_2 (10^{-10}–10^{-7}M) reduced the amplitude of the excitatory junction potentials (e.j.ps) evoked by field stimulation with no change in the membrane potential, input membrane resistance or the sensitivity of the muscle cells to acetylcholine (ACh).

5 LTC_4 (1.6×10^{-8}M) evoked a sustained contraction of the dog trachea; however, this agent did not affect either the amplitude of the twitch contractions or the e.j.ps evoked by field stimulation.

6 The amplitude of the e.j.p. was dependent on the external concentration of Ca^{2+}, and the inhibitory actions of $cTxA_2$ on e.j.ps were partly overcome by increasing the concentrations of $[Ca]_o$. When the amplitudes of e.j.ps were plotted against $[Ca]_o$ on a double log scale, the above relation yielded a straight line with a slope of 1.7 or 1.0, in the absence or presence of $cTxA_2$, respectively.

7 After treatment with Ca^{2+}-free 2 mM EGTA-containing solution, $cTxA_2$ or LTC_4 did not evoke a contraction in the dog trachea, whereas ACh (10^{-7}–10^{-6}M) did.

8 These results indicate that $cTxA_2$ and PGI_2 have dual actions on pre- and post-junctional membranes of the dog tracheal tissue, i.e. both agents inhibit the excitatory neuro-effector transmission in the dog trachea, presumably by inhibiting the release of ACh from the vagal nerve terminal. $cTxA_2$ and LTC_4 or PGI_2 evoke contraction or relaxation of the muscle tissue, respectively, apparently through direct actions on the smooth muscle cells.

Introduction

Prostaglandins play an important role in the regulation of airway smooth muscle tone. The prostaglandin E (PGE) series relax 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; Mathe *et al.*, 1971). The relaxations or constrictions elicited by the PGE or PGF series are not affected by atropine, mepyramine, methysergide or α- and β-adrenoceptor blocking agents, therefore, these events seem to have a direct action on the smooth muscle cells, possibly through a specific prostaglandin receptor (Mathe, 1976; Smith, 1976). There are chemicals which act as selective antagonists for the PGE or PGF series in a variety of smooth muscle preparations. For example, in the guinea-pig trachea and ileum, SC-19220 (1-acetyl-2-[8-chloro-10,11-dihydrobenz(b.f) (1.4) oxazepine-10-carbonyl] hydrazine) acts as a selective antagonist for PGE_2 or $PGF_{2\alpha}$, and blocks the direct actions of PGE or PGF series on

smooth muscle cells (Bennett & Posner, 1971; Farmer *et al.*, 1974). Thus, it is generally considered that the PGE or PGF series directly contract or relax the tracheobronchial smooth muscle cells.

However, low concentrations of the PGE or PGF series (10^{-12}–10^{-10}M) markedly reduce the amplitude of the twitch contractions and excitatory junction potentials (e.j.ps) evoked by activation of excitatory cholinergic nerve fibres in the dog trachea, with no change in the resting membrane potential, input membrane resistance and sensitivity of the muscle membrane to exogenous acetylcholine (Ito & Tajima, 1981a, b). The concentration of the PGE series required to produce relaxation of bronchial smooth muscle was in the range 10^{-9}–10^{-6}M. Thus, the nerve terminals of the cholinergic fibres are much more sensitive to prostaglandins than are the smooth muscle cells in the dog trachea.

If endogenous prostaglandins do play a physiological role in the regulation of the motility of tracheal muscle, the action would necessarily be mediated through prostaglandin receptors in the vagal nerve terminals. In support of this view, a prostaglandin antagonist, SC-19220, or prostaglandin synthesis inhibitor, indomethacin, reversed the decremental response of twitch contractions or e.j.ps evoked by nerve stimulations, and the former agent produced a sustained contraction of the dog tracheal tissue which was suppressed by atropine or PGE_2 (Inoue *et al.*, 1984). This evidence indicates that endogenous prostaglandins play an important role in inhibiting the release of acetylcholine from vagal nerve terminals, in both resting and active states.

Thromboxane A_2 (TxA_2), one of the products of enzyme action on the prostaglandin endoperoxides (PGG_2 and PGH_2) is a highly unstable but potent vaso- and bronchoconstrictor (Needleman *et al.*, 1976; Svensson *et al.*, 1977). Another product of enzyme action on PGG_2 and PGH_2 is prostacylcin (PGI_2), which has a number of characteristic actions including inhibition of blood coagulation and relaxation of vascular tissues (Armstrong *et al.*, 1978; Makita, 1983).

The actions of TxA_2 or PGI_2 on the pre-junctional nerve terminals in the airway smooth muscle tissues are poorly understood. We did comparative studies of the action of $cTxA_2$ and the sodium salt of PGI_2 on the pre- and post-junctional membrane of dog tracheal tissue. As TxA_2 and PGI_2 are unstable, synthetic carbocyclic thromboxane A_2 ($cTxA_2$) and the sodium salt of PGI_2 were used in place of the naturally occurring types of TxA_2 and PGI_2.

We also looked at the effects of leukotriene C_4 on neuro-effector transmission and on smooth muscle cells. Since metabolites of the cyclo-oxygenase pathway of arachidonic acid have a pre- and post-junctional action in dog tracheal tissue, it was of interest to observe the effects of lipoxygenase products in the arachidonic cascade on the pre- and post-junctional membrane.

Methods

Adult mongrel dogs of either sex, weighing 10–15 kg were anaesthetized with intravenous pentobarbitone (10–30 mg kg^{-1}). Segments of cervical trachea were excised and a dorsal strip of transversely running smooth muscle was separated from the cartilage. The mucosa and adventitial areolar tissues were carefully removed, under the microscope. The tracheal smooth muscle was cut in section, 2.0–2.5 mm wide and about 20 mm long for the double sucrose gap experiments. The preparation was bathed in a modified Krebs solution of 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 the pH was adjusted to 7.3–7.4. For intracellular recording of the membrane potential from single cells, strips of tissue 15–20 mm long, 1–2 mm wide and 0.3–0.4 mm thick were used. A conventional microelectrode filled with 3M KCl was inserted from the outer surface of the preparation. The chamber in which the muscle preparation was mounted had a volume of 2 ml, and was superfused at a rate of 3 ml min^{-1} at a temperature of 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 in detail elsewhere (Ito & Tajima, 1981a). To produce neurogenic responses, field stimulation was applied by a ring electrode placed in the centre pool of the apparatus. Single and repetitive stimulation was applied, with a current pulse of 50–100 μs 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. 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-5734) and the other end to a hook at the bottom of the bath. The strips were set up with an initial tension of 0.3 g and mechanical activity was recorded via a mechanotransducer on a pen recorder.

The following drugs were used, propranolol hydrochloride (Nikken Chemcial), indomethacin (Sigma), prostaglandin I_2 sodium salt (PGI_2; sodium salt), carbocyclic thromboxane A_2 ($cTxA_2$) and leukotriene C_4 (LTC_4; Ono), tetrodotoxin (Sankyo), FPL55712 (sodium 7- 3-(4-actyl-3-hydroxy- 2-propylphenoxy) 2-hydroxy propoxy-4-oxo-8 propyl- 4H-1-benzopyran-2-carboxylate (Fisons), acetylcholine (Sigma),

atropine sulphate (Daiichi) and ethylene glycol-bis (β-aminoethylether)-N-N'-tetraacetic acid (EGTA; Dozin).

Half-decay time of PGI_2 sodium salt was 1.2 h in glycine buffer solution, as estimated by bioassay of its hypotensive activities after intravenous injection in the anaesthetized dog (Kawasaki *et al.*, 1980). Therefore, freshly-prepared PGI_2-containing solutions were used in every experiment. $cTxA_2$, a stable analogue of thromboxane A_2, was an inhibitor of arachidonic acid-induced platelet aggregation and also inhibited thromboxane B_2 formation in rabbit platelets. Furthermore, $cTxA_2$ was 10,000 times more potent vasoconstrictor than TxB_2 in cat coronary artery.

Results (amplitude of contractions or e.j.ps) were expressed as mean ± s.d. and analyzed for significance using Student's *t* test.

Results

Effects of $cTxA_2$, PGI_2 and LTC_4 on the electrical membrane properties of the smooth muscle cells in the dog trachea

The effects of $cTxA_2$, PGI_2 or LTC_4 on the resting membrane potential were observed by use of the microelectrode method. Up to 10^{-7}M, $cTxA_2$, PGI_2 or LTC_4 had no effect on the resting membrane potential of smooth muscle cells of the dog trachea: control -60.5 ± 1.4 mV ($n = 50$); in $cTxA_2$ -60.7 ± 1.5 mV ($n = 30$); in PGI_2 -61.5 ± 1.5 mV ($n = 40$) and in LTC_4 -60.1 ± 1.6 mV ($n = 30$), respectively. With an increased concentration (10^{-6}M), $cTxA_2$ and LTC_4 slightly depolarized the membrane (about 2–3 mV), however the effect was not statistically significant. There were no apparent changes in the amplitude of electrotonic potentials, evoked by inward and outward current pulses applied extracellularly and recorded with a microelectrode, during application of $cTxA_2$ or LTC_4 10^{-7}M, thereby indicating that input membrane resistance of the smooth muscle cells was unaffected by $cTxA_2$ or LTC_4.

At 10^{-6}M, PGI_2 hyperpolarized the membrane from -60.7 ± 1.3 ($n = 30$) to -63.7 ± 1.6 ($n = 30$). However the amplitude of the electrotonic potential was little affected; the relative input membrane resistance measured after application of PGI_2 was 0.97 ± 0.07 times the control value ($n = 3$), (Figure 1).

Effects of $cTxA_2$, PGI_2 and LTC_4 on the twitch contractions evoked by nerve stimulation

To observe the effects of $cTxA_2$, PGI_2 or LTC_4 on the twitch contractions evoked by excitatory cholinergic nerve fibres in the dog tracheal tissue, field stimulation of short duration (500 μs) was applied in the presence of indomethacin (10^{-6}M) and propranolol (10^{-6}M) (Ito & Tajima, 1981a). The amplitude of the twitch contraction increased in proportion to the number of stimuli at a constant stimulus intensity and frequency (20 Hz), and was completely suppressed by pretreatment with either tetrodotoxin 2×10^{-7}M or atropine 10^{-6}M, indicating that the twitch responses are due to stimulation of excitatory cholinergic nerves.

Application of $cTxA_2$ (2.7×10^{-10}M–2.7×10^{-7}M) evoked a sustained increase in muscle tone and reduced the amplitude of twitch contractions. The amplitude of $cTxA_2$-induced contraction was dose-dependent, and the mean values were 5 ± 3.6 ($n = 5$),

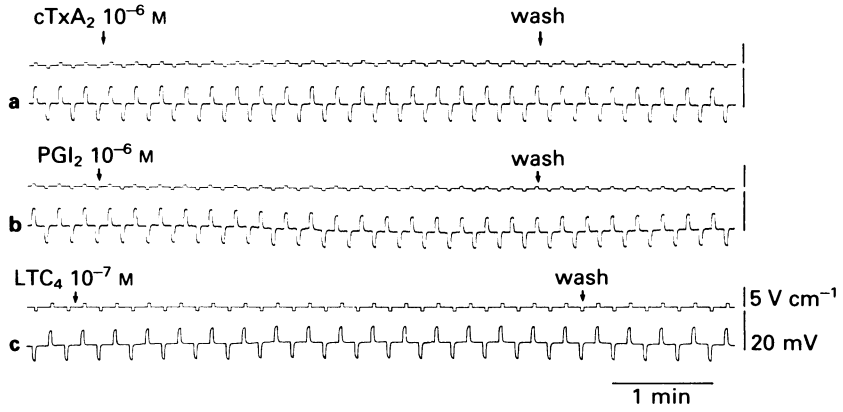

Figure 1 Effects of carbocyclic thromboxane A_2 ($cTxA_2$, 10^{-6}M), prostaglandin I_2 (PGI_2, 10^{-6}M) and leukotriene C_4 (LTC_4, 10^{-7}M) on the resting membrane potential and electrotonic potentials evoked by alternately applied inward and outward current pulses (2 s in duration). Upper trace in each pair of records indicates current injected into the muscle tissue, and lower trace the change in membrane potential of the smooth muscle cells recorded with microelectrode. Arrows indicate application and removal of the chemicals.

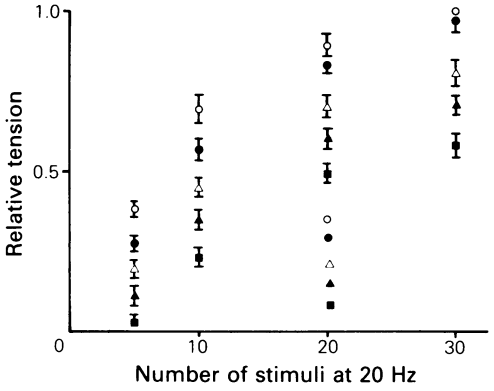

Figure 2 Effects of carbocyclic thromboxane A_2 ($cTxA_2$, 2.7×10^{-10}–2.7×10^{-7}M) on the amplitude of twitch contractions evoked by field stimulation with short duration. The amplitude of twitch contraction evoked by 30 stimuli at 20 Hz in normal Krebs solution was taken as a relative tension of 1.0. Repetitive field stimulation (5–30 stimuli at 20 Hz) were used to evoked twitch contractions. Control (○); $cTxA_2$ 2.7×10^{-10}M (●); 2.7×10^{-9}M (△); 2.7×10^{-8}M (▲) and 2.7×10^{-7}M (■).

7 ± 2.5 ($n = 4$), 11 ± 2.0 ($n = 3$) % of the amplitude of twitch contractions evoked by 30 stimuli at 20 Hz, when doses of 2.7×10^{-10}M, 2.7×10^{-9}M and 2.7×10^{-8}M $cTxA_2$ were applied. After application of atropine (10^{-6}M) or tetrodotoxin (2×10^{-7}M), the $cTxA_2$-induced contraction was still apparent (data not shown), indicating that the response was due to a direct action of $cTxA_2$ on the smooth muscle cells. Figure 2 shows the relationship between the amplitude of twitch tension and number of stimuli used to evoke twitch contractions in the presence or absence of $cTxA_2$ in various concentrations (2.7×10^{-10}M–2.7×10^{-7}M). $cTxA_2$ (2.7×10^{-10}M) inhibited the twitch contraction, when fewer than ten repetitive stimuli were given. In concentrations over 2.7×10^{-9}M, this agent suppressed, dose-dependently, the twitch contraction, under any stimulus condition used in the present experiments.

Contrary to the action of $cTxA_2$, PGI_2 ($> 10^{-7}$M) reduced the muscle tone and the amplitude of twitch contraction, dose-dependently. Figure 3 shows the relationship between the amplitude of twitch contractions in the presence or absence of various concentrations (2.7×10^{-7}M–2.7×10^{-6}M) of PGI_2.

Similar experiments were repeated using LTC_4 which at a concentration of 1.6×10^{-6}M increased the muscle tone; this was not affected by treatment with atropine (10^{-6}M) or tetrodotoxin (10^{-7}M), indicating that LTC_4 acts directly on smooth muscle cells of the dog trachea. However, LTC_4 had no effect on the amplitude of twitch contractions evoked by nerve stimulation in the concentrations up to 1.6×10^{-7}M.

Effects of $cTxA_2$, PGI_2 and LTC_4 on the amplitude of the excitatory junction potential (e.j.p.)

To assess the mechanisms involved in the inhibitory effect of $cTxA_2$ or PGI_2 on the twitch contraction evoked by field stimulation, effects of these compounds on e.j.ps were examined. To record e.j.ps and twitch contractions, the double sucrose gap method was used, and to obtain e.j.ps with a constant amplitude for a given stimulus condition, indomethacin (10^{-5}M) and propranolol (10^{-6}M) were applied throughout the experiments (Ito & Tajima, 1981a, b).

As shown in Figure 4, field stimulation (50 μs in duration) through electrodes placed in the centre pool of the double sucrose gap apparatus, produced e.j.p. followed by twitch contraction. $cTxA_2$ (10^{-10}M–10^{-7}M) reduced the amplitude of e.j.p. and twitch contractions dose-dependently, although the resting tension of the preparations was increased in proportion to the concentration of $cTxA_2$. In the presence of 10^{-10}M $cTxA_2$, the amplitude of the e.j.p. was decreased to $84 \pm 3\%$ (± s.d., $n = 7$) of the control value, and at 10^{-6}M, this agent completely blocked the generation of e.j.ps. When the amplitudes of e.j.ps were suppressed by various concentrations of $cTxA_2$ (10^{-10}M–10^{-6}M), there was no change in the membrane potential measured from single smooth muscle cell and in the membrane resistance of the smooth muscle cells, evaluated by the double sucrose

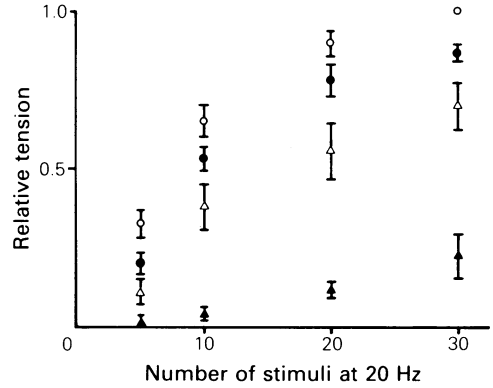

Figure 3 Relationship between the number of stimuli (5–30 at 20 Hz) used to evoke the twitch contraction and relative amplitude of the twitch in the presence or absence of prostaglandin I_2 (PGI_2, 2.7×10^{-7}–2.7×10^{-6}M). The amplitude of twitch contraction evoked by 30 stimuli at 20 Hz in normal Krebs solution was taken as a relative tension of 1.0. Control (○); PGI_2 2.7×10^{-7}M (●); 1.4×10^{-6}M (△) and 2.7×10^{-6}M (▲).

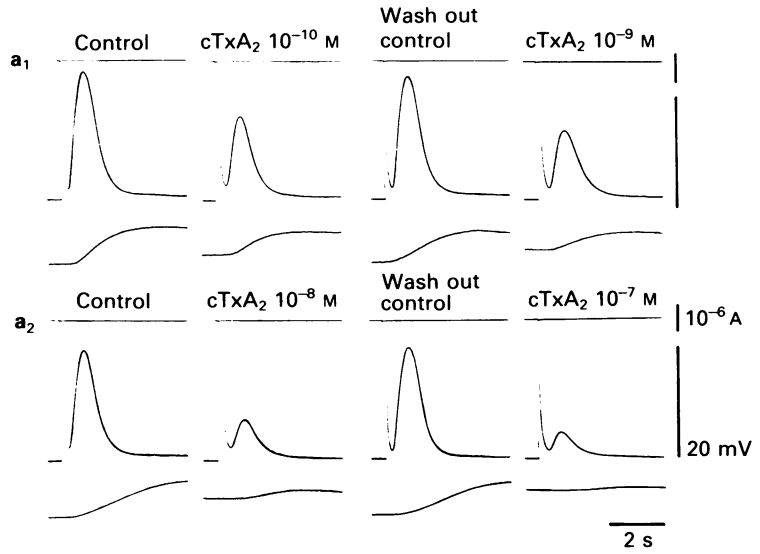

Figure 4 Effects of various concentrations of carbocyclic thromboxane A_2 ($cTxA_2$) on the amplitude of e.j.p. and phasic tension development. Single field stimulation (50 μs in duration) was applied to evoke e.j.p.

gap method. Figure 5a summarizes the effects of various concentrations of $cTxA_2$ on the amplitude of e.j.p, input membrane resistance and resting membrane potential.

Similar experiments were performed with PGI_2 (10^{-9}–10^{-6}M). PGI_2 (> 10^{-8}M) significantly reduced the amplitude of e.j.ps with no change in the membrane potential or input membrane resistance of the

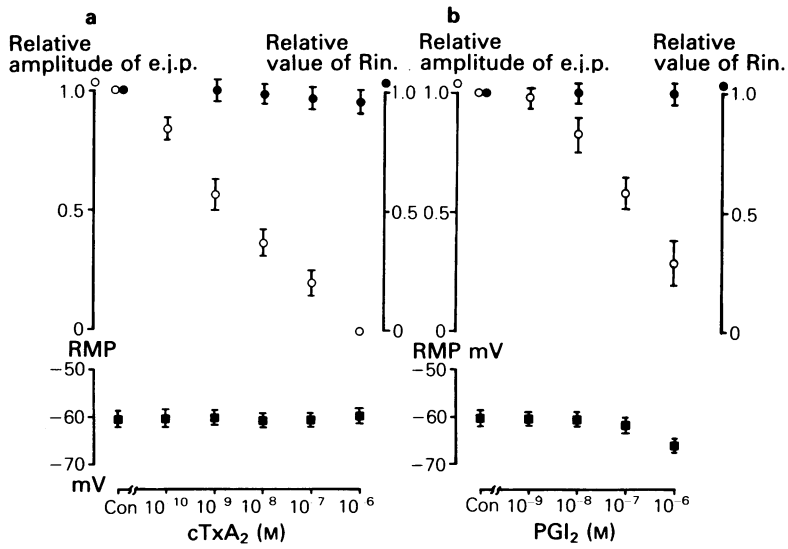

Figure 5 (a and b) Relationship between the concentration of carbocyclic thromboxane A_2 ($cTxA_2$) (a) or prostaglandin I_2 (PGI_2) (b) and the relative amplitude of e.j.p. (○), relative value of input membrane resistance (Rin.) (●) and resting membrane potential (RMP) (■) of the smooth muscle cells. The relative amplitude of e.j.p. in normal Krebs solution, and the relative amplitude of the electrotonic potentials produced by square wave pulses in normal Krebs solution (3 s in duration) was taken as 1.0. Each point is the mean value of 5–8 experiments, and vertical bars indicate 2 × s.d.

smooth muscle cells. Figure 5b shows the effects of various concentrations of PGI_2 (10^{-9}–10^{-6}M) on the relative amplitude of e.j.p. input membrane resistance and the membrane potential of the dog tracheal smooth muscle cells.

LTC_4 (10^{-8} and 10^{-7}M) had no effects on the amplitude of the e.j.ps.

Effects of $cTxA_2$ and PGI_2 on acetylcholine-induced contraction of the dog trachea

When the generation of e.j.ps was blocked completely by $cTxA_2$ or PGI_2, there was little change in the membrane potential or in the input membrane resistance of the smooth muscle cells. Therefore, we examined the effects $cTxA_2$ or PGI_2 on the sensitivity of muscle membrane to acetylcholine. For this purpose, the tension development induced by application of various concentrations of ACh was measured, before and during application of $cTxA_2$ or PGI_2.

$cTxA_2$ (2.7×10^{-8}M) or PGI_2 (1.4×10^{-6}M) did not affect the amplitude of the ACh-induced contraction (10^{-8}–10^{-3}M), although these agents significantly suppressed the amplitude of twitch contractions evoked by field stimulation. These data indicate that the ACh-sensitivity of the post-junctional smooth muscle is not affected by application of $cTxA_2$ or PGI_2.

Effects of $[Ca]_o$ on the e.j.p. and on actions of $cTxA_2$

Further to clarify the mechanisms involved in the drug action, the effects of $cTxA_2$ on the e.j.p. were observed in the presence of various concentrations of $[Ca]_o$ by the double sucrose gap method. When the log of the relative amplitude of e.j.p. was plotted against log $[Ca]_o$, straight lines with a slope of approximately 1.7 (1.65 ± 0.26 ($\pm$ s.d.) $n = 7$) were obtained, in the range from 0.5 to 2.5 mM $[Ca]_o$ (Figure 6). In the presence of $cTxA_2$, increasing $[Ca]_o$ produced an increase in the amplitude of e.j.p. which again followed a linear relationship. However, $cTxA_2$ (10^{-8} or 10^{-7}M) reduced the slope of the straight line to approximately 1 (1.10 ± 0.07 ($\pm$ s.d.), $n = 7$). Therefore, the interaction between $cTxA_2$ and $[Ca]_o$ is not one of simple competitive inhibition.

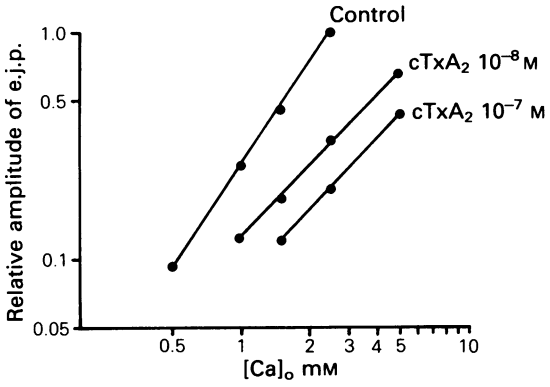

Figure 6 Effects of carbocyclic thromboxane A_2 ($cTxA_2$, 10^{-8}–10^{-7}M) on the relationship between Ca concentration and the relative amplitude of e.j.p. plotted on double-logarithmic scale. The relative amplitude of e.j.p. in 2.5 mM $[Ca]_o$ was given a relative amplitude of 1.0.

Effects of $[Ca]_o$ on the $cTxA_2$- or LTC_4-induced contraction

$cTxA_2$ (10^{-7}M) evoked a sustained contraction of the dog tracheal tissue (Figure 7a). The amplitude was about 10% of the amplitude of twitch contractions evoked by 10 stimuli at 20 Hz. The effects of $cTxA_2$ or LTC_4 were observed in Ca^{2+}-free EGTA (2 mM) containing solution. In this solution $cTxA_2$ did not increase the muscle tone, while ACh (5×10^{-7}M) evoked the contractions (Figure 7b).

Bath application of LTC_4 (10^{-7}M) evoked a sustained contraction of the muscle preparation in normal Krebs solution which was blocked by pretreatment with Ca^{2+}-free EGTA-containing solution for 10 min (Figure 7c) Figure 7d shows the effects of a rapid application of LTC_4 (10^{-6}M) on the muscle tone of the dog trachea in the presence of Ca^{2+}. LTC_4 (10^{-6}M) evoked a phasic contraction, the amplitude of which was about 40% of the amplitude of the contraction evoked by 10 stimuli at 20 Hz. After treatment with Ca^{2+}-free EGTA-containing solution, LTC_4 (10^{-6}M) did not modify the muscle tone, although ACh (10^{-7}M) did evoke a phasic contraction.

These results indicate that $cTxA_2$- or LTC_4-induced contractions are mainly due to an influx of Ca^{2+} across the muscle membrane and that ACh releases Ca^{2+} from the intracellular store sites.

Figure 7c also shows the effects of FPL55712, an antagonist for leukotrienes, on the LTC_4-induced contraction. Application of FPL55712 (10^{-5}M) evoked a phasic contraction. In the presence of FPL55712, LTC_4 10^{-7}M did not evoke a contraction and the amplitude of the 10^{-6}M LTC_4-induced contraction was reduced to approximately 20% of the control value.

Effects of PGI_2 on excess $[K]_o$-induced contraction

Contrary to the effects of $cTxA_2$ or LTC_4, PGI_2 relaxed the muscle tone of the dog trachea. However, PGI_2 did not affect the amplitude of ACh-induced contraction. Therefore, it was of interest to observe the effects of PGI_2 on the excess $[K]_o$-induced contracture.

Figure 8 shows the effects of PGI_2 (5×10^{-7} or 5×10^{-6}M) on the 20.2 mM or 39.0 mM $[K]_o$-induced

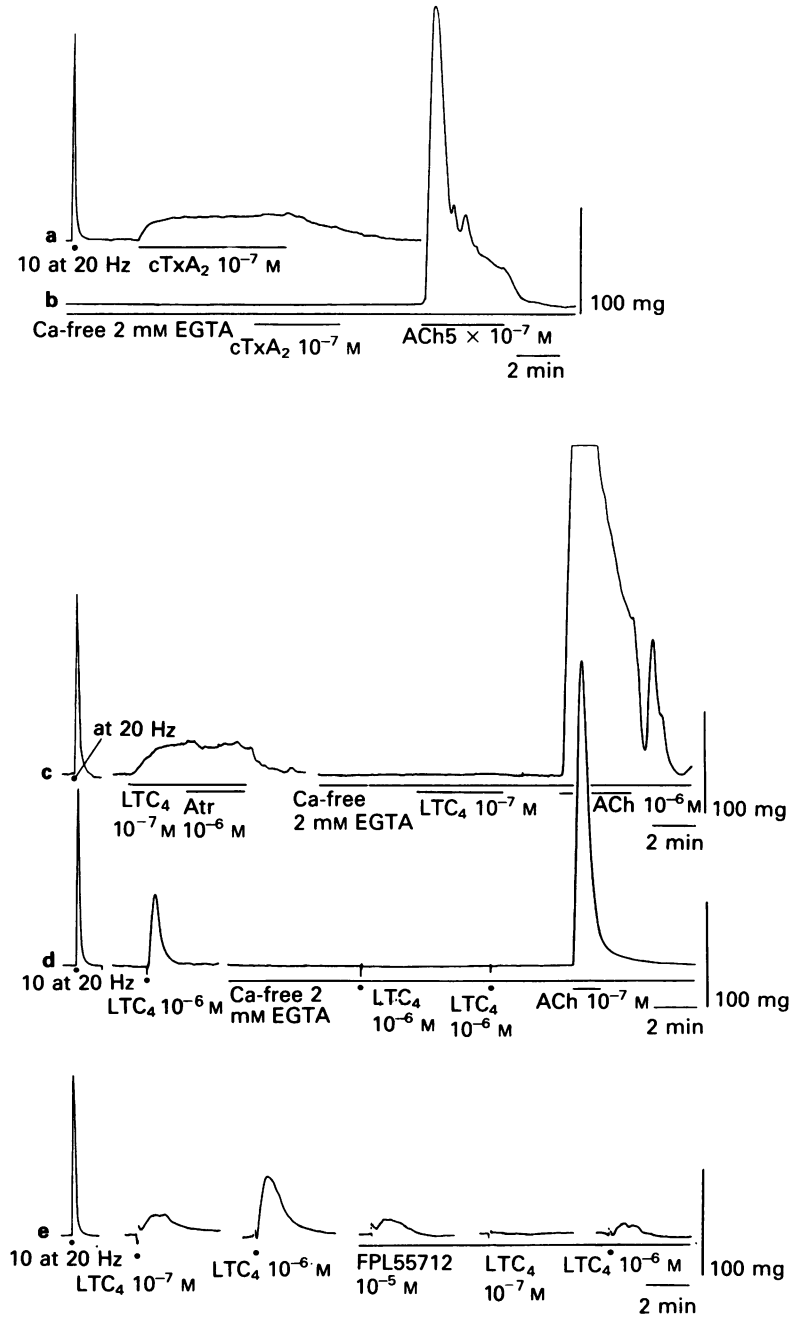

Figure 7 Effects of carbocyclic thromboxane A_2 ($cTxA_2$) (a and b) or leukotriene C_4 (LTC_4) (c–e) on the muscle tone during the treatment of the tissue with Ca-free 2 mM EGTA containing solution. (a and b) Effects of $cTxA_2$ before (a) and during treatment of the tissue with Ca-free 2 mM EGTA solution; (c) effects of bath applications of LTC_4 (10^{-7}M) on the muscle tone before and during application of Ca-free 2 mM EGTA containing solution; (d) effects of bolus applications of LTC_4 (10^{-6}M) on the muscle tone before and during application of Ca-free 2 mM EGTA containing solution; (e) effects of bolus applications of LTC_4 (10^{-7} or 10^{-6}M) in the presence or absence of FPL55712 (10^{-5}M). Dots indicate the application of field stimulation (10 stimuli at 20 Hz). Horizontal bars indicate the application of various chemicals or Ca-free 2 mM EGTA containing solution.

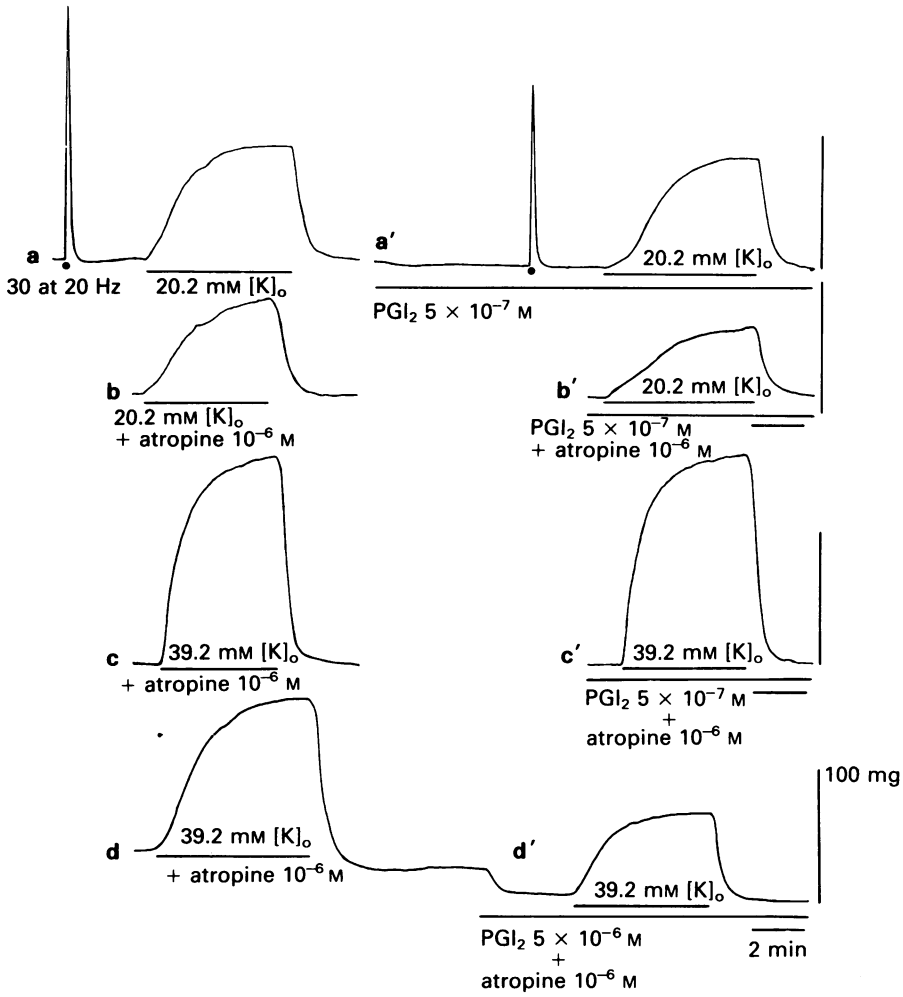

Figure 8 Effects of prostaglandin I_2 (PGI_2) (5×10^{-7}M or 10^{-6}M) on the excess $[K]_o$ (20.2 mM or 39.2 mM)-induced contraction of the dog trachea in the presence or absence of atropine (10^{-6}M). (a and a'). Twitch contractions evoked by field stimulation (30 stimuli at 20 Hz) or excess $[K]_o$ (20.2 mM) in the absence (a) or presence (a') of PGI_2 (5×10^{-7}M). Note PGI_2 suppressed the amplitude of twitch contractions evoked by field stimulation, but did not affect the amplitude of excess-$[K]_o$ induced contraction; (b and b'); similar experiments were carried out in the presence of atropine (10^{-6}M); (c and c') effects of PGI_2 on 39.2 mM $[K]_o$-induced contraction in the presence of atropine (10^{-6}M). PGI_2 did not suppress the amplitude of excess $[H]_o$-induced contraction; (d and d') increased concentrations of PGI_2 (10^{-6}M) reduced the resting tension and amplitude of the excess $[K]_o$-induced contraction, to 55% of the control value.

contraction, in the presence or absence of atropine (10^{-6}M).

PGI_2 (5×10^{-7}M) reduced the muscle tone and the amplitude of the twitch contraction evoked by field stimulation (10 stimuli at 20 Hz) to 70% of the control value. However, the amplitude of 20.2 mM $[K]_o$-induced contraction was not affected (Figure 8a & a'). After pretreatment of the muscle tissue with atropine (10^{-6}M), the amplitude of the 20.2 mM $[K]_o$-induced contraction was reduced to 75 ± 5 ($n = 5$) % of the control value. In the presence of atropine, PGI_2 (5×10^{-7}M) further reduced the amplitude of contraction evoked by 20.2 mM $[K]_o$ containing solution to 55 ± 4 ($n = 5$) % of the control value (Figure 8b & b'). However, when the increased concentration of $[K]_o$ (39.2 mM) was used to evoke the contraction in the presence of 10^{-6}M atropine, 5×10^{-7}M PGI_2 did not (Figure 8c & c') suppress the amplitude of excess $[K]_o$-induced contraction. An increase in the concentration of PGI_2 (5×10^{-6}M) reduced the amplitude of the contraction evoked by 39.2 mM $[K]_o$ to about 50% of the control value (Figure 8d & d').

Discussion

The direct actions of TxA_2 or PGI_2 on the vascular or airway smooth muscle cells has been given most attention (see for example, Svensson *et al.*, 1977; Moncada & Vane, 1979; Moncada, 1982).

Our results obtained with dog tracheal tissue clearly show that $cTxA_2$ or PGI_2 act not only directly on airway smooth muscle cell but also indirectly through release of ACh from the vagus nerve terminal.

The PGE or PGF series also act on the pre- and post-junctional membrane in the dog tracheal tissue (Ito & Tajima, 1981a, b), and there is a 1000 fold difference in the concentration of PGE series effective in the pre- and post-junctional actions. Thus, PGE_1 and PGE_2 at 10^{-12}M significantly reduced transmitter release from the vagus nerve terminal, thereby reducing the amplitude of twitch contractions or e.j.ps evoked by field stimulation, and at 10^{-9}M both agents showed direct inhibitory actions on the smooth muscle cells (Inoue *et al.*, 1984). Furthermore, the primary prostanoids, $cTxA_2$ and PGI_2 act on pre- and post-junctional membrane in the smooth muscle tissues of the guinea-pig mesenteric artery (Kuriyama & Makita, 1982; Makita, 1983).

Thus, the roles of $cTxA_2$ and PGI_2 as modulators in excitatory neuroeffector transmission cannot be ruled out when interpreting drug actions. LTC_4 had no effect on the neuro-effector transmission in our experiment.

The inhibitory actions of $cTxA_2$ or PGI_2 on excitatory neuro-effector transmission were counteracted by application of a high concentration of $[Ca]_o$. It has been reported that prostaglandins interact with extracellular Ca^{2+} at the activated nerve terminals, thereby reducing the amount of transmitter released by adrenergic or cholinergic nerve fibres (Ito & Tajima, 1979; 1981a; Kuriyama & Makita, 1982), and that in the guinea-pig mesenteric artery, $cTxA_2$ or PGI_2 consistently inhibited the excitatory neuro-effector transmission, presumably through inhibition of noradrenaline release by suppression of Ca^{2+} influx at the nerve terminal (Makita, 1983). The precise mechanisms involved in the action of $cTxA_2$ on the nerve terminal is unknown, however $cTxA_2$ may modify the Ca^{2+} channel through conformational changes of the membrane structure, or it may render intracellular Ca^{2+} inactive, thereby inducing a reduction in the transmitter release.

LTC_4 or $cTxA_2$ evoked a sustained contraction in the presence of atropine or tetrodotoxin. Since our experiments were carried out in the presence of indomethacin, the action of LTC_4 on smooth muscle cells of the dog trachea is not caused by production of TxA_2 or other cyclo-oxygenase products which may be released by LTC_4 (Piper & Samhoun, 1982). Furthermore, FPL55712, an antagonist for leukotrienes, abolished the action of LTC_4 on the smooth muscle cells. Parenchymal strips of guinea-pig lung were more sensitive to LTC_4 than were larger tissues from airways such as the isolated trachea (Piper & Samhoun, 1982). However, contractions of parenchymal strips elicited by LTC_4 or LTD_4 were greatly reduced by imidazole and carboxyhepthylimidazole, both potent and specific inhibitors of thromboxane synthetase (Lewis & Watts, 1982). This indicates that LTC_4 exerts an action in parenchymal strips mainly via generation of the potent bronchoconstrictor, TxA_2, while the tracheal tissue produces mainly prostaglandin-like materials (Gryglewski *et al.*, 1976). This would explain the difference in the potency of LTC_4 in contraction of the parenchymal strips of the lung (at above 10^{-12}M; Holme *et.*, 1980) and trachea (at above 10^{-10}M in the guinea-pig; Sirois *et al.*, 1981, and at above 10^{-8}M in the dog trachea).

In the guinea-pig ileum, contractile responses to slow reacting substance A (SRS-A) were inhibited by Ca^{2+} withdrawal from the bathing fluid and by a calcium antagonist, methoxyverapamil, thereby suggesting that Ca^{2+} influx is involved in the action of leukotrienes (Findlay *et al.*, 1981). Similarly, in the dog trachea, LTC_4 or $cTxA_2$ evoked no mechanical responses after pretreatment of the tissue with Ca^{2+}-free EGTA containing solution. On the contrary, acetylcholine or caffeine (Ito & Itoh, 1984a, b) evoked a sustained contraction in Ca^{2+}-free EGTA containing solution, indicating that acetylcholine or caffeine releases Ca^{2+} from intracellular Ca^{2+} storage sites.

PGI_2, on the other hand, suppressed the excess $[K]_o$-induced contraction but not the ACh-induced contracture. This indicates that PGI_2 does not inhibit Ca^{2+} release from the intracellular storage sites by the action of acetylcholine, but does inhibit the Ca^{2+} influx across the cell membrane evoked by excess $[K]_o$. In the trachea, Ca stored in the cells probably plays a more important role in the initiation of contraction (Ito & Itoh 1984a & b).

All of the primary prostaglandins tested showed an inhibitory action on transmitter release from the cholinergic and adrenergic nerve terminals (Ito & Tajima, 1979; 1981a; Kuriyama & Makita, 1982; Makita, 1983). On the other hand, PGE series or PGI_2 relaxed, and PGF series or $cTxA_2$ contracted the smooth muscle cells in the airway.

The precise mechanism involved in the inhibitory and excitatory actions of PGI_2, $cTxA_2$ or LTC_4 on the pre- and post-junctional membrane remain to be determined. However, PGI_2 or $cTxA_2$ may interact with $[Ca]_o$ at activated nerve terminals, and on the other hand $cTxA_2$ or LTC_4 may act as a calcium ionophore at the membrane of the smooth muscle cells (Serhan *et al.*, 1982).

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