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Effects of prostaglandin I_2 and carbocyclic thromboxane A_2 on smooth muscle cells and neuromuscular transmission in the guinea-pig mesenteric artery

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1 In the guinea-pig mesenteric arteries neither prostacyclin (PGI_2) nor carbocyclic thromboxane A_2 ($cTxA_2$) affected membrane potential in concentrations below 1×10^{-6} M. Increasing the concentration to 3×10^{-6} M either slightly hyperpolarized or depolarized the membrane with little change in membrane resistance.

2 At a concentration of 1×10^{-7} M, the amplitude of the first e.j.p. and the enlarged amplitudes of the subsequent e.j.ps evoked by trains of stimuli were reduced consistently by PGI_2 or $cTxA_2$. Facilitation was unaffected by either agent.

3 The inhibitory actions of PGI_2 were partly overcome by increased concentrations of 5 mM $[Ca]_o$ and were accelerated by a reduced concentration of 1.25 mM $[Ca]_o$.

4 The amplitude of the contraction evoked by perivascular nerve stimulation was inhibited to a greater extent by PGI_2 than by $cTxA_2$ at concentrations below 1×10^{-6} M.

5 The contraction evoked by 5×10^{-6} M noradrenaline (NA) or excess concentrations of 20.2 mM $[K]_o$ was enhanced by 1×10^{-8} M – 1×10^{-6} M $cTxA_2$ and suppressed by 1×10^{-8} M – 1×10^{-6} M PGI_2. The minimum concentration of $cTxA_2$ required to produce the contraction was 1×10^{-8} M.

6 These results indicate that transmission at the neuromuscular junction was inhibited consistently by PGI_2 or $cTxA_2$, presumably due to inhibition of NA release by suppression of the Ca influx at the nerve terminals. Whereas PGI_2 inhibited, $cTxA_2$ enhanced the mechanical response by a direct action on the smooth muscle cells.

Introduction

The unstable but potent vasoconstrictor and vasodilator prostaglandin intermediates thromboxane A_2 (TxA_2) and prostacyclin (prostaglandin I_2; PGI_2), respectively have been detected in vascular and other tissues. TxA_2, originally discovered by Samuelsson and his colleagues (Hamberg, Svensson & Samuelsson, 1975), aggregated platelets and caused vasoconstriction, while PGI_2 inhibited blood coagulation and relaxed vascular tissue (Armstrong, Lattimer, Moncada & Vane, 1978). These actions were attributed to a direct effect on vascular smooth muscle (Samuelsson, 1976; Moncada & Vane, 1979; Hemler, Cook & Lands, 1979).

Prostaglandins themselves relax (PGE_1 and PGE_2) or contract ($PGF_{2\alpha}$) vascular smooth muscle directly. In the rabbit pulmonary artery, PGE_1 or PGE_2 above 1×10^{-7} g/ml hyperpolarize the membrane, reduce membrane resistance and relax the muscle; $PGF_{2\alpha}$ depolarizes the membrane, reduces membrane resistance and contracts smooth muscle (Kitamura, Suzuki & Kuriyama, 1976). However, the direct actions of these primary prostaglandins on the guinea-pig mesenteric artery were observed only at high concentrations above 3×10^{-6} M (Kuriyama & Makita, 1982a).

Microelectrode and isometric tension recording methods have been used in the present investigation to examine the effects of PGI_2 and TxA_2 on neuromuscular transmission and directly on the smooth muscle in the guinea-pig mesenteric artery. As PGI_2 and TxA_2 are unstable, it is difficult to examine the physiological effects on smooth muscle reactivity using electrophysiological methods. In the present work, synthetic carbocyclic TxA_2 ($cTxA_2$) and the sodium salt of PGI_2 were used instead of the naturally occurring types of TxA_2 and PGI_2. $cTxA_2$

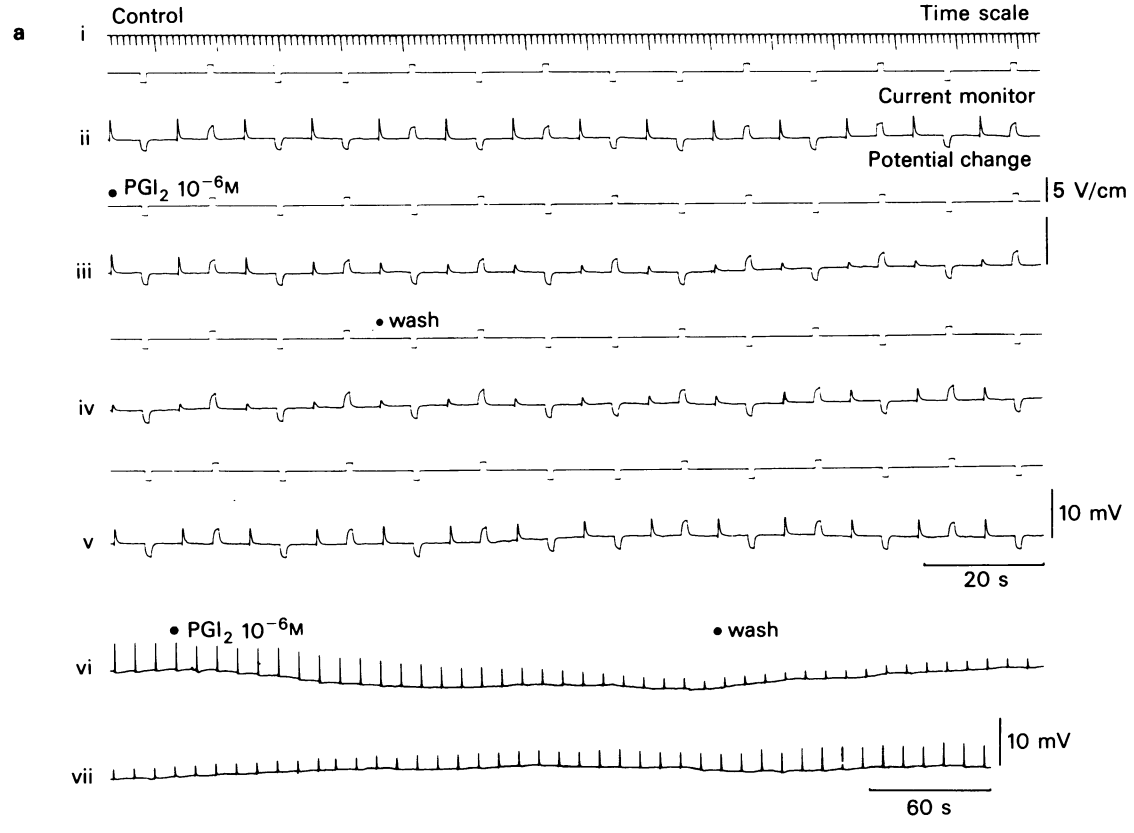
produces vasoconstriction and the sodium salt of PGI_2 has an inhibitory action on platelet aggregation (Lefer, Smith, Araki, Smith, Aharony, Claremon, Magolda & Nicolaou, 1980; Kawasaki, Ishii, Wakitani & Tsuboshima, 1980; Smith, Lefer, Aharony, Smith, Magolda, Claremon & Nicolaou, 1981; Towart & Perzborn, 1981).

Methods

Guinea-pigs (300–400 g) were stunned, exsanguinated and the mesenteric artery with parallel lymph and veins removed from the mesenteric vascular bed of the jejunum. Arteries, approximately 3–4 mm long and 0.2–0.3 mm wide were mounted in a 2 ml organ bath.

To stimulate the perivascular nerves (pulse duration 0.03–0.05 ms supramaximal voltage) and the muscle membrane (pulse duration 1.2 s), the partition stimulation method (Abe & Tomita, 1968) was used. In some experiments, the nerves were stimulated by placing a pair of AgCl-coated, 0.05 mm diameter silver wires directly on the arterial tissue. The electrical response of the membrane was measured with glass capillary microelectrodes filled with 3 M KCl; the electrode resistance was between 80–100 MΩ. The microelectrode was inserted into the arterial muscle cell from the outer surface. The tissue was superfused at 2 ml/min with Krebs solution at 35°–36°C (Bülbring, 1954) of the following composition, (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.0 and glucose 11.5. The solution was bubbled with 97% O_2 and 3% CO_2; the pH was maintained at 7.2–7.3.

Since muscle cells of the mesenteric artery are arranged circularly, isometric contractions were recorded from ring preparations (0.3–0.5 mm in diameter, 1–1.5 mm wide). Two fine 50 μm diameter steel needles were inserted into the lumen. The end of one needle was connected to a micromanipulator in order to suspend the immersed tissue and the end of the other needle to a mechanotransducer to record the contraction (Nihon Kohden, MZ 3A) (Itoh, Kuriyama & Suzuki, 1981). To stimulate smooth muscle directly (pulse duration, 1 s or more) in the presence of 3×10^{-7} M tetrodotoxin (TTX) or perivascular nerves (pulse duration, 0.1 ms, sup-



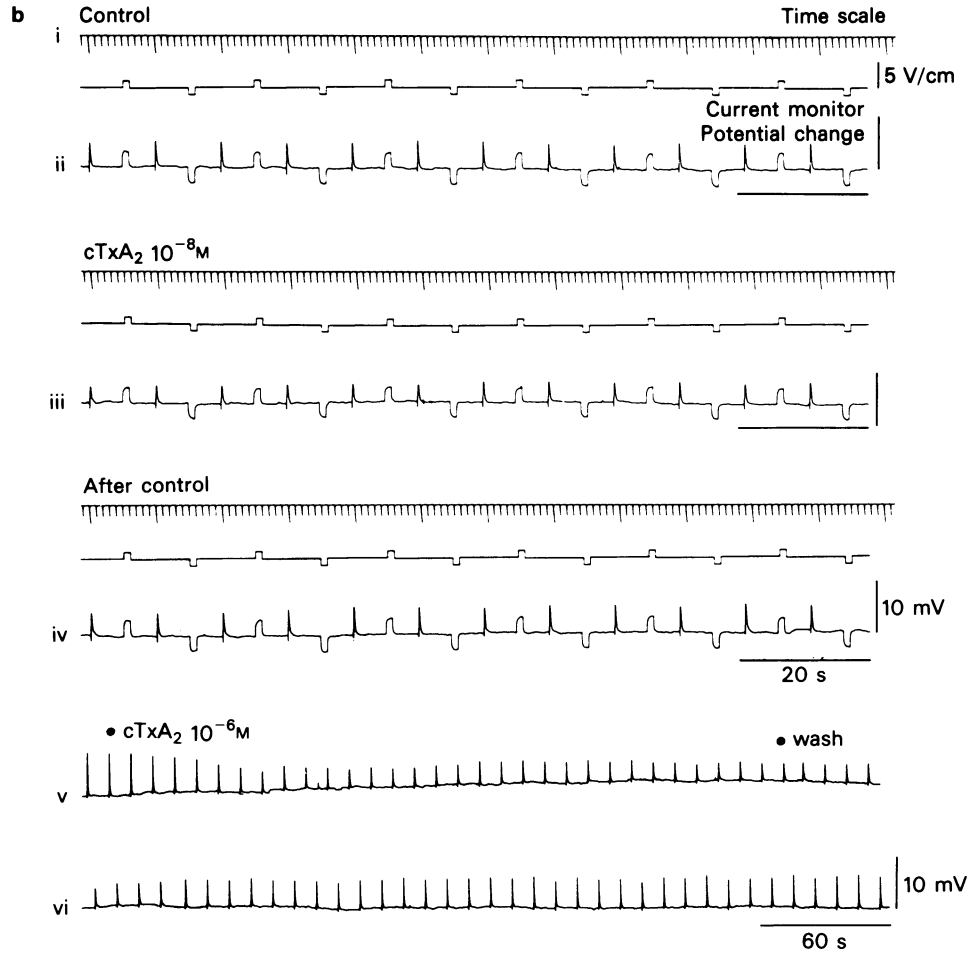


Figure 1 Effects of prostacyclin (PGI_2) and carbocyclic thromboxane A_2 ($cTxA_2$) on the amplitude of the e.j.p. evoked by perivascular nerve stimulation (0.05 ms pulse duration, supramaximal voltage) and on electrotonic potential evoked by alternately applied inward and outward current pulses (1.2 s pulse duration). (a) Effects of PGI_2 (1×10^{-6} M): (i) – (v) continuous records; (vi), (vii) continuous records. (b) Effects of $cTxA_2$ (1×10^{-6} M). The perivascular nerve stimulation (0.1 Hz supramaximal voltage) was applied before, during and after application of PGI_2 or $cTxA_2$.

ramaximal voltage), a pair of AgCl-coated, silver plates were placed in parallel with and on either side of the immersed tissue (Kuriyama & Makita, 1982a).

The following drugs were used at the molar concentrations indicated in the results; prostaglandin I_2 (PGI_2 sodium salt) and carbocylic thromboxane A_2 ($cTxA_2$; Ono), tetrodotoxin (TTX: Sankyo), (−)-noradrenaline (NA; Sigma, to prevent destruction of NA, ascorbic acid was added).

The solution of NA was freshly prepared for each experiment.

All values are the mean ± s.d. of the indicated number of microelectrode penetrations or preparations.

Results

Effects of prostacyclin and carbocyclic thromboxane A_2 on resting membrane potential

The resting membrane potential of smooth muscle cells from the guinea-pig mesenteric artery was -69.6 ± 1.4 mV ($n = 35$); the membranes were electrically quiescent. When PGI_2 or $cTxA_2$ was applied at concentrations of less than 1×10^{-6} M, the membrane potential did not change, with 3×10^{-6} M PGI_2, the membrane was hyperpolarized from -69.4 ± 1.9 mV ($n = 15$) to -71.9 ± 2.1 mV ($n = 20$; $P < 0.05$). With application of 3×10^{-6} M

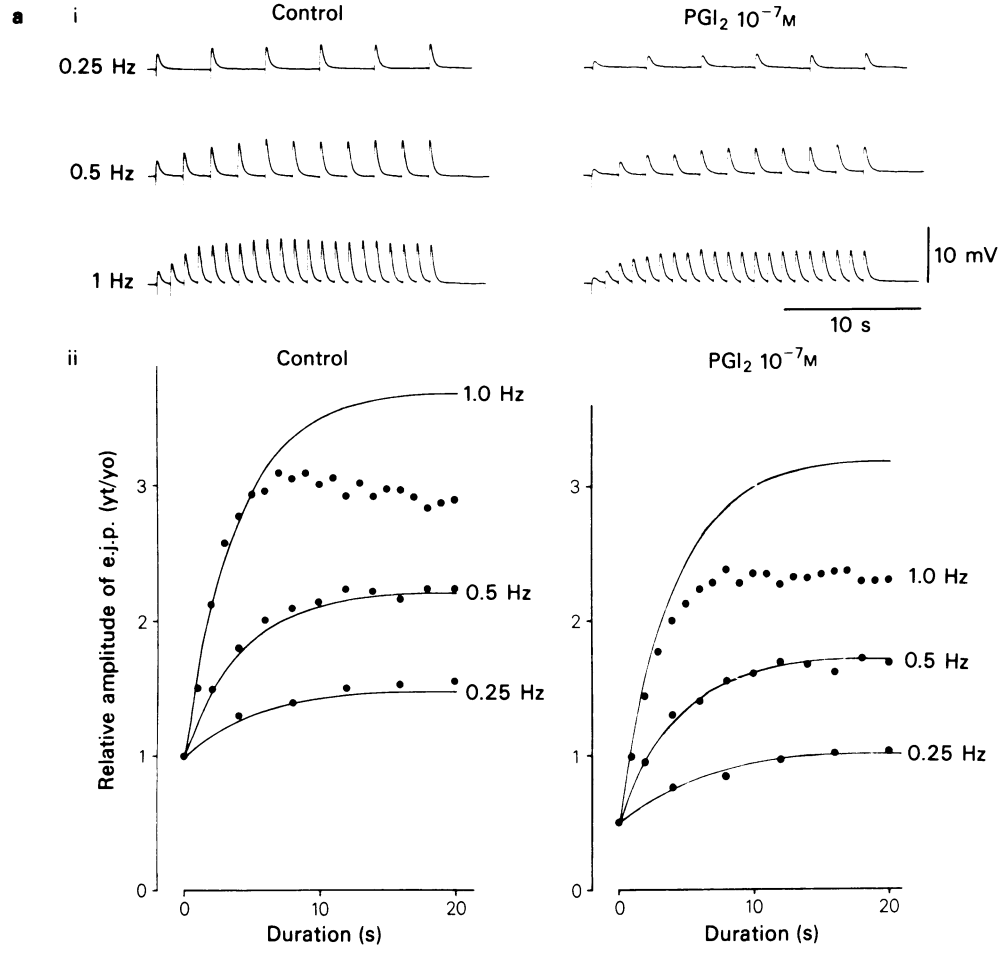
$cTxA_2$, the membrane was depolarized from -69.0 ± 1.6 mV ($n = 15$) to -66.1 ± 1.8 mV ($n = 20$; $P < 0.05$).

The amplitude of the electrotonic potential was not affected by application of PGI_2 1×10^{-6} M, and there was little change in membrane potential (the relative input resistance measured before and during application of PGI_2 was 0.96 times the control, $n = 3$, (the amplitude of the electrotonic potential in the presence of agent/the amplitude of electrotonic potential in Krebs solution)[2] (Abe & Tomita, 1968)). The length constant of tissue was reported to be 0.8 mm (Kuriyama & Suzuki, 1981). The input resistance was not affected by application of 1×10^{-6} M $cTxA_2$, with little change in membrane potential (relative input resistance was 0.98 times the control, $n = 4$).

There was no change in the current-voltage relationships before and during application of PGI_2 (1×10^{-6} M) or $cTxA_2$ (1×10^{-6} M) recorded at 0.1 mm from the stimulating electrode.

Effects of prostacyclin and carbocyclic thromboxane A_2 on the e.j.p. evoked by perivascular nerve stimulation

The e.j.p. evoked by perivascular nerve stimulation (0.05 ms pulse; supramaximal voltage) was abolished by 3×10^{-7} M tetrodotoxin. When perivascular nerve stimulation was applied between applications of inward and outward current pulses (1.2 s pulse duration) (Figure 1 a(iii)), the amplitude of the e.j.p. was reduced gradually by application of 1×10^{-6} M PGI_2 with no change in the amplitude of the electrotonic potential. Removal of PGI_2 restored the amplitude of the e.j.p. Figure 1 a(ii) shows the effects of PGI_2 on the e.j.p. evoked by perivascular nerve stimulation at 0.1 Hz. A slight increase in membrane potential and a reduction in the amplitude of the e.j.p. occurred; restoration of the e.j.p. to the control value following removal of PGI_2 required a much longer time than did the recovery of the membrane potential. $cTxA_2$



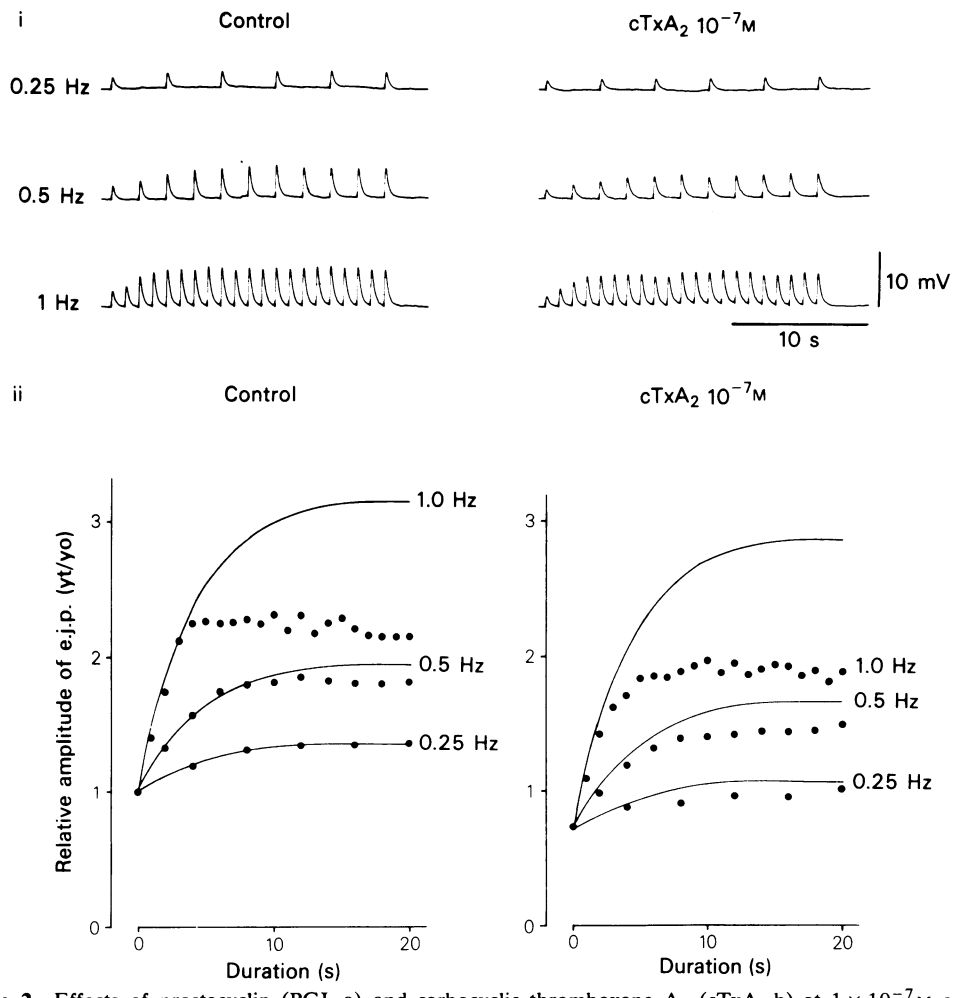


Figure 2 Effects of prostacyclin (PGI_2,a) and carbocyclic thromboxane A_2 ($cTxA_2$,b) at 1×10^{-7} M on the facilitation of e.j.ps evoked by repetitive perivascular nerve stimulation (0.05 ms, supramaximal voltage) at 0.25, 0.5 and 1.0 Hz. (a) (i) changes in the amplitude of e.j.ps during short trains of pulses at three different frequencies before and during application of PGI_2; (ii) continuous curves are the predicted relationship, as given in the text. Vertical axis; relative amplitude of e.j.p. The amplitude of first e.j.p. (yo) was assigned the value 1.0 Horizontal axis; duration of a train stimulation with different frequencies. Dots are measured values produced by three different frequencies of stimulation. (b) The amplitude of e.j.ps during facilitation before and during application of $cTxA_2$. The experimental procedures were the same as those observed in (a).

(1×10^{-6} M) reduced the amplitude of e.j.p. (Figure 1b(i) (v) (vi)) with little change of membrane potential. The effects of $cTxA_2$ on the e.j.p. were also reversible. The minimum concentration of PGI_2 or $cTxA_2$ required to reduce the amplitude of the e.j.p. was 1×10^{-10} M and 1×10^{-8} M, respectively.

Stimulation at frequencies above 0.2 Hz facilitated the e.j.p. which, then reached a steady level. The amplitude of the e.j.ps after facilitation, depended on the stimulus frequency. At three different stimulus frequencies, the amplitude of the e.j.p. evoked by the first of a series of stimuli in the presence of PGI_2 was lower than that in the control, however, the facilitation process remained apparent (Figure 2a). In Figure 2a(ii), the amplitude of the e.j.p. evoked by the first in a series of stimuli at the indicated frequencies was recorded as a relative amplitude of 1.0. After application of 1×10^{-7} M PGI_2, the amplitude of the first e.j.p. was reduced to 0.5 times the control value ($n = 5$), however, the exponential growth curve of the e.j.ps was still apparent, at any given frequency of stimulation.

To investigate the activity of PGI_2 or $cTxA_2$ on the mobilization of Ca^{2+} in the nerve terminals, their effects on the facilitation of the amplitude of the e.j.ps during trains of stimulation were studied. This facilitation prosess could be predicted by the method of Mallart & Martin (1967). Changes in the amplitude of the e.j.p. in normal Krebs solution evoked at different intervals after application of the conditioning stimuli were plotted on a log scale against the time interval. Changes in the amplitude of the e.j.ps produced by the conditioning stimulation could by classified into two components; the first occurred in

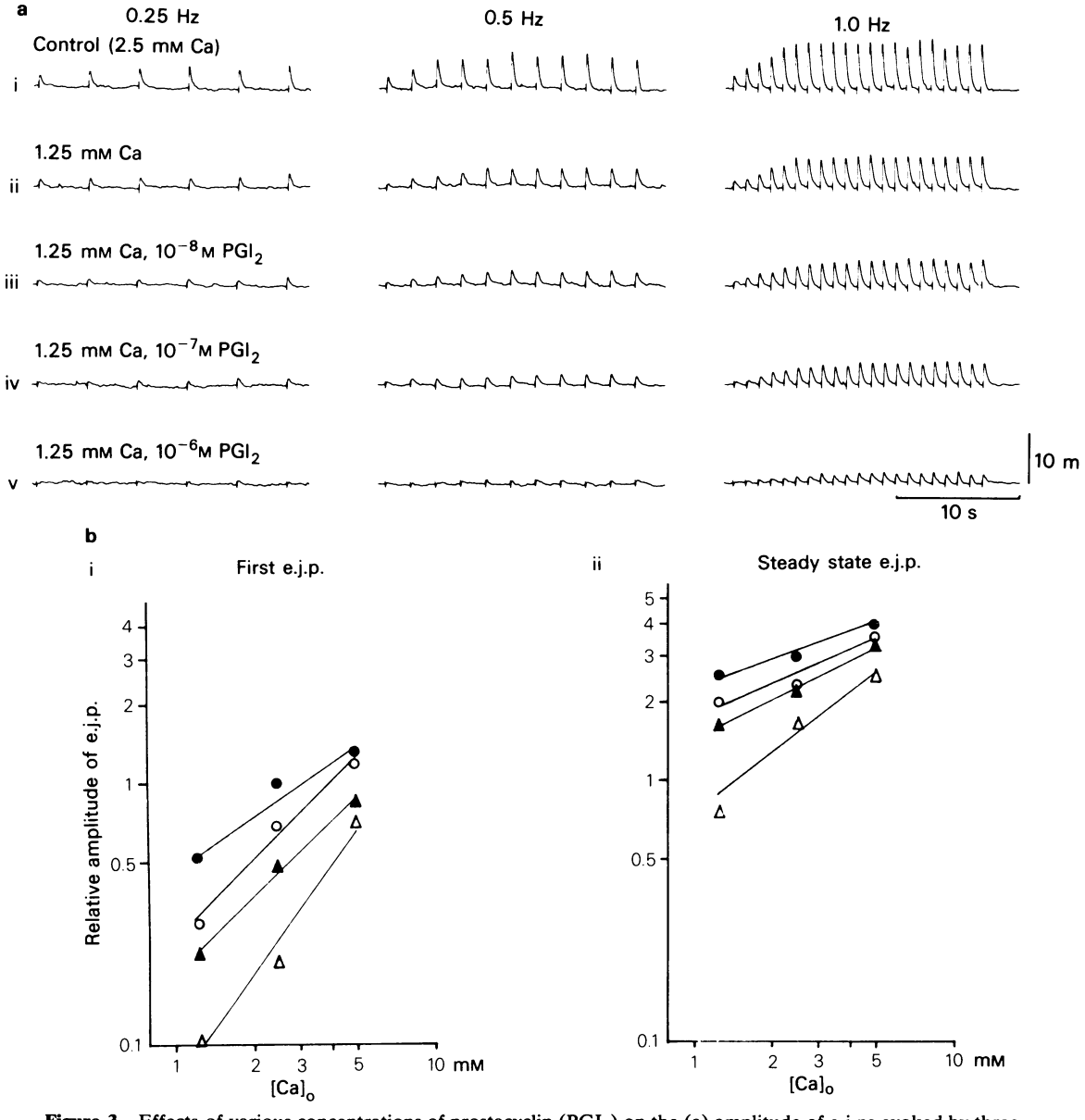


Figure 3 Effects of various concentrations of prostacyclin (PGI_2) on the (a) amplitude of e.j.ps evoked by three different stimulus frequencies of perivascular nerve stimulation in 1.25 mM $[Ca]_o$ and on the (b) relationship between $[Ca]_o$ and the relative amplitude of the first (b(i)) and subsequently generated steady amplitude (b(ii)) of e.j.ps plotted on double logarithmic scales. The amplitude of the first e.j.p. recorded in 2.5 mM $[Ca]_o$ was assigned the value 1.0. In (b) control (●); PGI_2 10^{-8} M (○), 10^{-7} M (▲) and 10^{-6} M (△).

response to stimuli given at short (a few hundred ms) intervals and the second at intervals of over 1.0 s. To predict the growth in the amplitude of the e.j.ps at a stimulus frequency of less than 1.0 Hz, the second component was used to calculate the rate constant of decay of the curve, i.e. if facilitation (f) is introduced as $y-y_o/y_o$, where y_o is the amplitude of the first e.j.p., and y is the amplitude of a test e.j.p., then the facilitation could be expressed as $f = f_1 e^{-bt}$. Here f_1 is the theoretical facilitation at zero time and b is the rate constant of decay of the curve. The values obtained for b and f_1 were 0.8 and 0.256, respectively ($n = 3$). These parameters were inserted into the equation, $f = f_1(e^{b\Delta t} - 1)^{-1}(1 - e^{-b\Delta t})$, thereby expressing the facilitation process. The continuous lines in Figure 2a(ii) represent the predicted relationship using the rate constant measured in Krebs solution in the presence or absence of PGI_2 (1×10^{-7} M). These results indicate that the recorded e.j.ps and the predicted facilitation curves roughly agree except for an extended stimulation at 1.0 Hz. Thus the facilitation can be explained by the same mechanism as that in skeletal muscles attributed to a mobilization of Ca in nerve terminals (see Katz, 1969).

Similar experimental procedures were used to observe the effects of $cTxA_2$ on the amplitude of the e.j.p. and the facilitation process. In the presence of 1×10^{-7} M $cTxA_2$, the amplitude of the first e.j.p. was reduced to 0.73 times the control, but the facilitation process was not affected at any frequency between 0.25 Hz – 1.0 Hz.

In Krebs solution, the amplitude of the e.j.ps depended on the $[Ca]_o$ concentration, an increase in $[Ca]_o$ enhanced, while a decrease reduced both the amplitude of the first e.j.p. and those after completion of the facilitation process (Kuriyama & Makita, 1982a,b). With a low concentration of $[Ca]_o$ 1.25 mM, the amplitude of the first e.j.p. and that of those after facilitation was completed were reduced consistently. Additions of various concentrations of PGI_2 inhibited the amplitude of the e.j.ps in a dose-dependent fashion (Figure 3a). Figure 3b shows the effects of three different concentrations of PGI_2 or $cTxA_2$ on the e.j.ps in three different concentrations of $[Ca]_o$ at the stimulus frequency of 1.0 Hz ($n = 5$). In these experiments, the amplitude of the e.j.p. evoked by the first in a series of stimuli was assigned the value 1.0 (Figure 3b(i)). To clarify that PGI_2 competes with Ca^{2+} influx at presynaptic nerve terminals, the relationship between the amplitude of the first e.j.p. and the Ca concentration was plotted on a log-log scale. In the presence or absence of PGI_2 ($1 \times 10^{-8} - 1 \times 10^{-6}$ M) there was no parallel relationship between $[Ca]_o$ and the amplitude of the first e.j.p., or between $[Ca]_o$ and the steady amplitude of the e.j.p. after completion of the facilitation process.

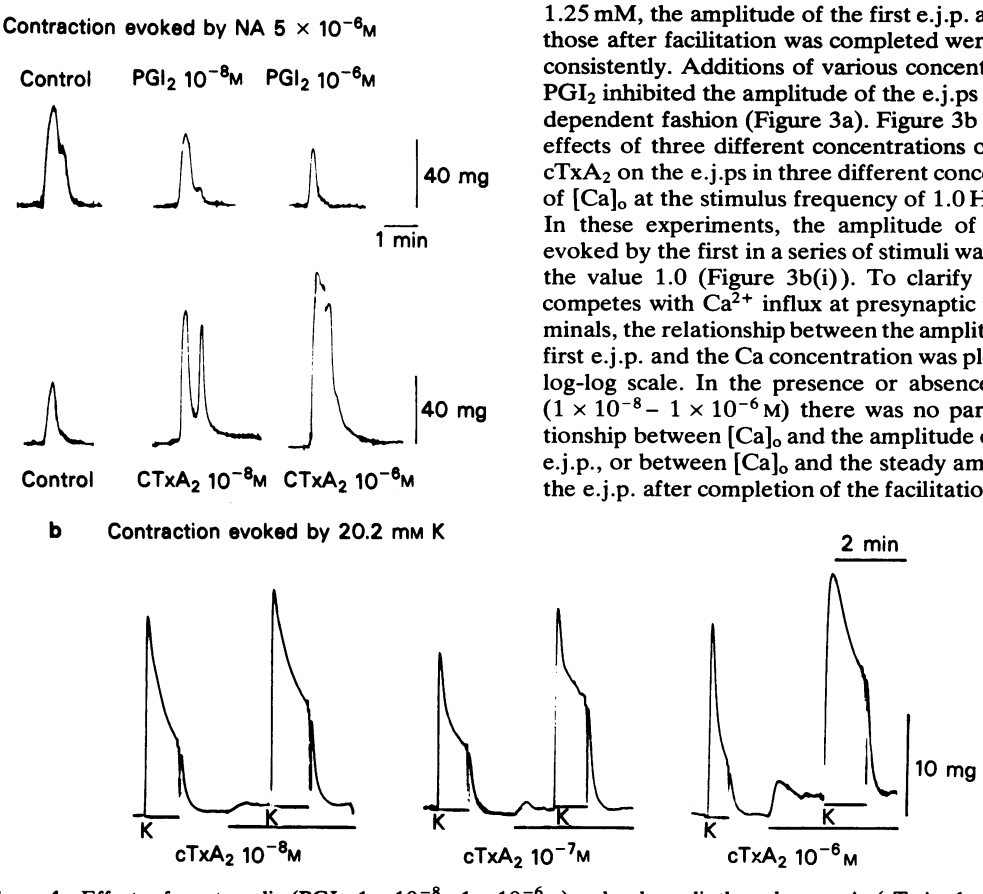


Figure 4 Effects of prostacyclin (PGI_2, $1 \times 10^{-8} - 1 \times 10^{-6}$ M) and carbocyclic thromboxane A_2 ($cTxA_2$, $1 \times 10^{-8} - 1 \times 10^{-6}$ M) on the mechanical responses evoked by (a) 5×10^{-6} M noradrenaline (NA), and (b) the effects of $cTxA_2$ (1×10^{-8} M – 1×10^{-6} M) on the 20.2 mM $[K]_o$ induced contraction. PGI_2 or $cTxA_2$ was simultaneously applied with 5×10^{-6} M NA. In (b) after application of 20.2 mM $[K]_o$, $cTxA_2$ was applied before and during application of 20.2 mM $[K]_o$.

These results indicate that while the PGI_2-induced suppression of the e.j.p. is partly restored by adding excess $[Ca]_o$, the action of PGI_2 and $[Ca]_o$ on the amplitude of e.j.p. is not competitive.

Effects of prostacyclin and carbocyclic thromboxane A_2 on the mechanical responses evoked by nerve stimulation or by chemicals

Figure 4 shows the effects of PGI_2 and $cTxA_2$ on the mechanical responses evoked by application of 5×10^{-6} M NA (a) and 20.2 m M $[K]_o$ (b). The amplitude of the NA-induced contraction was enhanced 3.2 times by 1×10^{-6} M $cTxA_2$, and reduced to 0.45 ± 0.14 of the control by 1×10^{-6} M PGI_2 ($n = 4$). The minimum concentration of $cTxA_2$ required to produce the contraction was 1×10^{-8} M (b). The contraction evoked by 20.2 mM $[K]_o$ was also enhanced by $cTxA_2$ in concentrations over 1×10^{-8} M. However, enhancement of the mechanical response by $cTxA_2$ was much more evident in the case of the contraction evoked by NA than that evoked in the excess $[K]_o$ solution.

Figure 5 shows the effects of PGI_2 and $cTxA_2$ on the mechanical response evoked by perivascular nerve stimulation (0.1 ms pulse duration; 20 Hz and 20 pulses, supramaximal voltage). Both PGI_2 and $cTxA_2$ consistently inhibited the amplitude of contraction. The inhibition of the contraction by PGI_2 appeared greater (0.22 ± 0.1 times the control by 1×10^{-6} M, $n = 5$; $P < 0.05$) than that by $cTxA_2$ (0.62 ± 0.1 times the control by 1×10^{-6} M, $n = 5$; $P < 0.05$).

Discussion

TxA_2, an unstable metabolic product of arachidonic acid, is a potent vasoconstrictor *in vitro*, in human coronary artery strips (Piper & Vane, 1969; Ellis, Oelz, Roberts, Payne, Sweetman, Nies & Oates, 1976; Needleman, Minks & Raz, 1976; Needleman, Kulkarni & Raz, 1977; Kulkarni, Wang & Eakins, 1979; Wang, Kulkarni & Eakins, 1980). PGI_2, also an unstable metabolite of arachidonic acid in vascular tissues, has potent antiplatelet aggregating and vasodilator properties (Moncada, Glyglewski, Bunting & Vane, 1976a,b; Moncada, Needleman, Bunting & Vane, 1976; Johnson, Lincoln, Nidy, Schneider, Thompson & Axen, 1978).

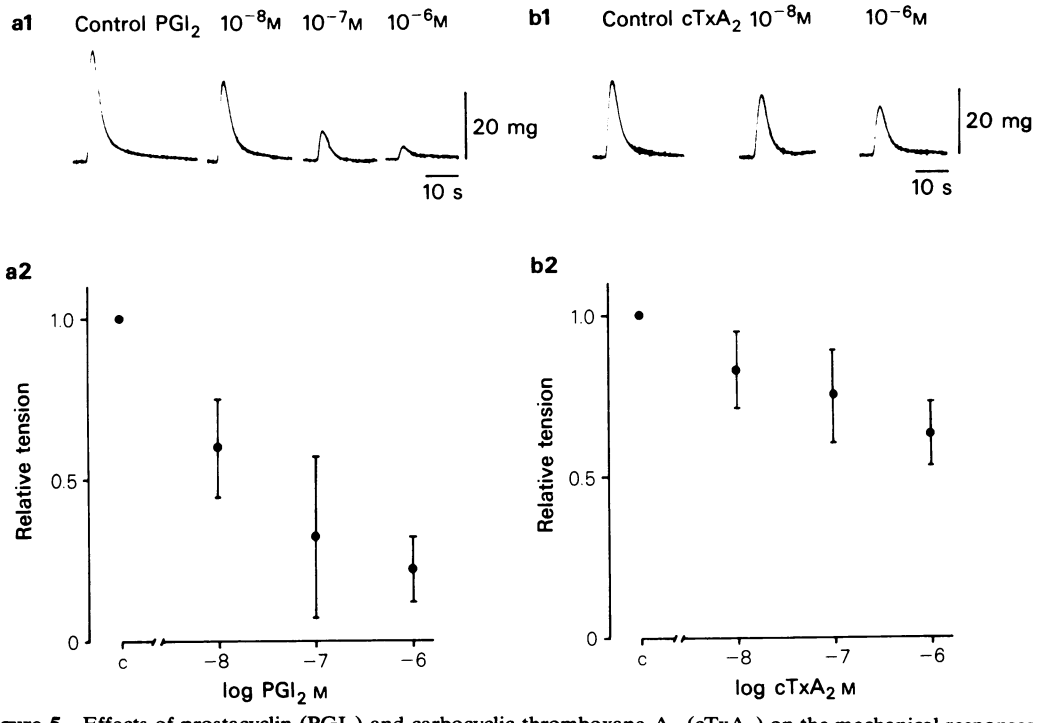


Figure 5 Effects of prostacyclin (PGI_2) and carbocyclic thromboxane A_2 ($cTxA_2$) on the mechanical responses evoked by perivascular nerve stimulation 0.1 ms, 20 Hz, 20 pulses PGI_2 or $cTxA_2$ was applied just before application of perivascular nerve stimulation. Vertical bars in a2 and b2 indicate $2 \times$ s.d. ($n = 4$). The amplitude of the contraction evoked by perivascular nerve stimulation before application of PGI_2 or $cTxA_2$ was registered as a relative tension of 1.0

$cTxA_2$ and the sodium salt of PGI_2 were used instead of TxA_2 and PGI_2, respectively. The PGI_2 sodium salt is less potent than PGI_2 but is more potent than PGE_1 with regard to the anti-aggregating activity of platelets (Kawasaki *et al.* 1980). PGI_2 increases coronary blood flow (Kaley, Messina, Hintze, Roberts, Martin & Slomiary, 1977) and decreases the blood flow in the mesenteric and gracilis muscles but does not affect the femoral blood flow (Svensson & Fredholm, 1977; Dusting, Chapple, Hughes, Moncada & Vane, 1978). $cTxA_2$, a structural analogue of TxA_2 constricts rabbit basilar and saphenous arteries and isolated coronary vessels (Lefer *et al.*, 1980; Nicolaou, Magolda & Claremon, 1980; Towart & Perzborn, 1981). $cTxA_2$ does not induce platelet aggregation (Smith et al., 1981) and inhibits the actions of endoperoxide and arachidonic acid-induced aggregation of platelets (Lefer *et al.*, 1980).

In the guinea-pig mesenteric artery, PGI_2 inhibited the amplitude of the first e.j.p. but not the facilitation process and suppressed the amplitude of the contraction evoked by perivascular nerve stimulation, NA and higher concentrations of $[K]_o$. These actions could lead to a vasodilatation. $cTxA_2$ inhibited the first e.j.p. but not the facilitation process. The contraction evoked by perivascular nerve stimulation was inhibited but the contraction evoked by NA and higher concentrations of $[K]_0$ were enhanced markedly. $cTxA_2$ may, therefore, inhibit a nerve mediated contraction, but enhance the other exogenous contraction such as that produced by the higher concentrations of $[K]_o$ or NA.

In the guinea-pig mesenteric artery, the nature of the adrenoceptor differs in the extra-junctional and junctional regions on the smooth muscle membrane; for example, the former was inhibited by prazosin and phentolamine, but the latter was not (Kuriyama & Makita, 1982b). This receptor was termed the gamma receptor. Such differences of postsynaptic α-adrenoceptors were also observed in the case of saphenous artery and the intra-intestinal mesenteric arterioles (Holman & Surprenant, 1979; 1980; Hirst & Neild, 1980; 1981). The inhibition of the e.j.p. evoked by perivascular nerve stimulation in this vascular muscle was not related to changes in the input resistance of the membrane. It is not yet certain whether PGI_2 or $cTxA_2$ inhibits the sensitivity of α-adrenoceptors distributed in the junctional region or whether the release of NA from nerve terminals is inhibited. Probably these inhibitory actions on the e.j.p. were considered to be the presynaptic inhibition of the transmitter release as suggested for the action of primary prostaglandins. Thus all prostaglandin tested (PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2 and $cTxA_2$) suppress neuromuscular transmission in the mesenteric artery, and to some extent, these inhibitory actions are restored by application of high concentrations of $[Ca]_o$. The influx of Ca during transmitter release from nerve terminals is probably inhibited, as suggested for the action of the primary prostaglandins (Kuriyama & Makita, 1982a). In the facilitation process, discrepancies observed between measured and predicted values after several stimuli at 1.0 Hz may be due to activation of the negative feedback mechanism mediated by presynaptic α_2-adrenoceptors by increased release of NA (Kuriyama & Makita, 1982b). Both PGI_2 and $cTxA_2$ did not affect the discrepancies between the measured and predicted values of the e.j.p.. Therefore, both agents did not interfere with presynaptic α_2-adrenoceptors to modify the NA release.

Inhibition of the amplitudes of the first e.j.ps and of those after completion of the facilitation process during treatment with $cTxA_2$ and PGI_2 were similar, yet inhibition of the mechanical responses evoked by perivascular nerve stimulation by PGI_2 was greater than by $cTxA_2$. Although $cTxA_2$ inhibits the NA release from nerve terminals, the amount of NA released following repetitive perivascular nerve stimulation increases and NA diffuses beyond the neuromuscular junctional area. $cTxA_2$ may enhance the sensitivity of extra-junctional receptors to NA. The increased sensitivity of these receptors for NA may result in restoration of the inhibition of neurally-induced contraction following the decrease of released transmitters. This speculation could explain the reduced inhibitory effect of $cTxA_2$ on the contraction evoked by perivascular nerve stimulation.

In the mesenteric artery, application of 20.2 mM $[K]_o$ depolarized the membrane from −59.2 mV to −49.8 mV, and application of 5×10^{-6} M NA depolarized the membrane to −51.4 mV (Takata, 1980). PGI_2 or $cTxA_2$ (below 1×10^{-6}M) did not modify the membrane potential and membrane resistance in the resting state, but may do so in the activated state. As a result, either inhibition or enhancement of the contraction evoked by NA or higher $[K]_o$ may occur.

$cTxA_2$ itself at a concentration of 1×10^{-8} M produced only a small contraction but markedly enhanced that produced by NA and to a lesser extent, K. Whether $cTxA_2$ and PGI_2 modify the Ca influx at the surface membrane or modify the release of the stored Ca in the cell was not elucidated. However, if $cTxA_2$ acts as a vasoconstrictor agent, it may accelerate the contraction evoked by a direct action on the vascular smooth muscles, but not that evoked by the perivascular nerve stimulation. PGI_2 may inhibit, indirectly, the contraction evoked by activation of the perivascular nerves and, directly evoked by activation of the vascular smooth muscle.

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References

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.*, **196,** 87–100.

AMSTRONG, J.M., LATTIMER, N., MONCADE, S. & VANE, J.R. (1978). Comparison of vasodepressor effects of prostacyclin and 6-oxo-prostaglandin $F_{1\alpha}$ with those of prostaglandin E_2 in rats and rabbits. *Br. J. Pharmac.*, **62,** 125–130.

BÜLBRING, E. (1954). Membrane potentials of smooth muscle fibers of taenia coli of the guinea-pig. *J. Physiol.*, **125,** 302–305.

DUSTING, G.J., CHAPPLE, D.J., HUGHES, R., MONCADA, S. & VANE, J.R. (1978). Prostacyclin induces coronary vasodilatation in anesthesized dogs. *Cardiovascular Res.*, **12,** 720–730.

ELLIS, E.F. OELZ, O., ROBERTS, L.J., PAYNE, N.A., SWEETMAN, B.J., NIES, A.S. & OATES, J.A. (1976). Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A_2. *Science*, **193,** 1135–1137.

HAMBERG, M., SVENSSON, J. & SAMUELLSON, B. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci. U.S.A.*, **72,** 2994–2998.

HEMLER, M.E., COOK. H.W. & LANDS, W.M. (1979). Prostaglandin biosynthesis can be triggered by lipid peroxides. *Arch. Biochem. Biophys.*, **193,** 340–345.

HIRST, G.D.S. & NEILD, T.O. (1980). Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. *Nature*, **283,** 767–768.

HIRST, G.D.S. & NEILD, T.O. (1981). Localization of specialized noradrenaline receptors at neuromuscular junctions on arterioles of the guinea-pig. *J. Physiol.*, **313,** 343–350.

HOLMAN, M.E. & SURPRENANT, A.M. (1979). Some properties of the excitatory junction potentials recorded from saphenous artery of rabbits. *J. Physiol.*, **287,** 337–351.

HOLMAN, M.E. & SURPRENANT, A.M. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71,** 651–661.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1981). Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. *J. Physiol.*, **321,** 513–535.

JOHNSON, R.A., LINCOLN, F.H., NIDY, E.G., SCHNEIDER, W.D., THOMPSON, J.L. & AXEN, V. (1978). Synthesis and characterization of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$, prostaglandin I_1 and prostaglandin I_3. *J. Am. Chem. Soc.*, **100,** 7690–7705.

KALEY, G., MESSINA, E.J., HINTZE, T.H., ROBERTS, A.M., MARTIN, E.G. & SLOMIARY, B.L. (1977). Effects of the metabolites of arachidonic acid on coronary blood flow (Abstr). *Prostaglandins*, **13,** 1011.

KATZ, B. (1969). *The Release of Neural Transmitter Substances.* Liverpool: University Press.

KAWASAKI, A., ISHII, K., WAKITANI, K. & TSUBOSHIMA, M. (1980). Comparison of the activities of prostacyclin and its stable analogue on the platelet aggregation and cardiovascular systems. *In Advances in Prostaglandin and Thromboxane Research*, vol. 6. ed. Samuelsson b. & Paoletti, R. pp. 331–336. New York: Raven Press.

KITAMURA, K., SUZUKI, H. & KURIYAMA, H. (1976). Prostaglandin action on the main pulmonary artery and portal vein of the rabbit. *Jap. J. Physiol.*, **26,** 681–692.

KULKARNI, P.S., WANG, H.H. & EAKINS, K.E. (1979). Pharmacological behavior of isolated human and dog coronary arteries (Abstr). *Proc. 4th Int. Conf. Prostagl.*, 64.

KURIYAMA, H. & MAKITA, Y. (1982a). Modulation of neuromuscular transmission by endogenous and exogenous prostaglandins in the guinea-pig mesenteric artery. *J. Physiol.*, **327,** 431–448.

KURIYAMA, H. & MAKITA, Y. (1982b). Modulation of noradrenergic transmission in the guinea-pig mesenteric artery – an electrophysiological study – *J. Physiol.*, (in press).

KURIYAMA, H. & SUZUKI, H. (1981). Adrenergic transmission in the guinea-pig mesenteric artery and their cholinergic modulations. *J. Physiol.*, **317,** 383–396.

LEFER, A.M., SMITH, E.F.III., ARAKI, H., SMITH, J.B., AHARONY, D., CLAREMON, D.A., MAGOLDA, R.L. & NICOLAOU, K.C. (1980). Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carbocyclic thromboxane A_2, a stable analog of thromboxane A_2. *Proc. natn. Acad. Sci. U.S.A.* **77,** 1706–1710

MALLART, A, & MARTIN, A.R. (1967). An analysis of facilitation of transmitter release at the neuromuscular junction of the frog. *J. Physiol.*, **193,** 679–694.

MONCADA, S., GRYGLEWSKI, R.J., BUNTING, S. & VANE, J.R. (1976a). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature,* **263,** 663–665.

MONCADA, S., GRYGLEWSKI, R.J., BUNTING, S. & VANE, J.R. (1976b). A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. *Prostaglandins*, **12,** 715–733.

MOCADA, S., NEEDLEMAN, P., BUNTING, S. & VANE, J.R. (1976). Prostaglandin endoperoxides and thromboxane generating systems and their selective inhibition. *Prostaglandins*, **12,** 323–325.

MONCADA, S. & VANE, J.R. (1979). The role of prostacyclin in vascular tissue. *Fedn Proc.*, **38,** 66–71.

NEEDLEMAN, P., KULKARNI, P.S. & RAZ, A. (1977). Coronary tone modulation: Formation and actions of prostaglandin endoperoxides and thromboxanes. *Science*, **195,** 409–412.

NEEDLEMAN, P., MINKES, M. & RAZ, A. (1976). Thromboxanes: selective biosynthesis and distinct biological properties. *Science*, **193,** 163–165.

NICOLAOU, K.C., MAGOLDA, R.L. & CLAREMON, D.A. (1980). Carbocyclic thromboxane A_2. *J. Am. Chem. Soc.*, **102,** 1404.

PIPER, P.J. & VANE, J.R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature,* **223,** 29–35.

SAMUELSSON, B. (1976). *Advances in Prostaglandin and Thromboxane Research.* ed. Samuelsson, B. & Paoletti, R. New York: Raven Press.

SMITH, E.F.III., LEFER, A.M., AHARONY, D., SMITH, B.J.,

MAGOLDA, R.L., CLAREMON, D. & NICOLAOU, K.C. (1981). Carbocyclic thromboxane A_2: aggregation of myocardial ischemia by a new synthetic thromboxane A_2 analog. *Prostaglandins*, **21,** 443–456.

SVENSSON, J. & FREDHOLM, B.B. (1977). Vasoconstrictor effect of thromboxane A_2 *Acta physiol. scand.*, **101,** 366.

TAKATA, Y. (1980). Regional differences in electrical and mechanical properties of guinea-pig messenteric vessels. *Jap. J. Physiol.*, **30,** 709–728.

TOWART, R. & PERZBORN, E. (1981). Nimodipine inhibits carbocyclic thromboxane-induced contractions of cerebral arteries. *Eur. J. Pharmac.*, **69,** 213–215.

WANG, H.H., KULKARNI, P.S. & EAKINS, K.E. (1980). Effects of prostaglandins and thromboxane A_2 on the coronary circulation of adult dog and puppies. *Eur. J. Pharmac.*, **66,** 31–41.

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