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MECHANISM OF NORADRENALINE POTENTIATION BY PROSTAGLANDIN E_2 IN RAT MESENTERIC ARTERY

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1 Effects of prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGF_{2\alpha}$) on vasoconstrictor responses to noradrenaline (NA) and methoxamine in isolated mesenteric arteries of the rat were investigated.

2 PGE_2 and to a lesser extent $PGF_{2\alpha}$ potentiated vasoconstrictor responses to NA and methoxamine.

3 Prior treatment with reserpine increased, and bretylium reduced, the extent of potentiation significantly.

4 NA vasoconstriction persisted for 1 h after Ca^{2+} was removed from the perfusing Krebs solution. Prostaglandin-induced potentiation was absent in Ca^{2+}-free Krebs, but increased proportionately with increase in external Ca^{2+} concentration.

5 Vasoconstriction induced by high potassium, was not potentiated by PGE_2.

6 It is concluded that PGE_2 potentiates NA vasoconstriction by facilitating Ca^{2+} influx.

Introduction

Potentiation of the contractile responses of vascular smooth muscles to biogenic amines may be due to inhibition of enzymatic degradation of the amine (Kalsner 1969a; 1970a; Levin & Furchgott, 1970), inhibition of uptake (Kalsner & Nickerson, 1969a; Kalsner & Frew, 1972) or interference with the rebinding of calcium released into the environment of the contractile elements during contraction (Kalsner, 1970c). Prostaglandins enhance responses to stimulants in both the visceral and vascular smooth muscles. In concentrations smaller than those needed to contract the guinea-pig myometrium, prostaglandin E_1 (PGE_1) increased responses to vasopressin, acetylcholine and calcium chloride (Eagling, Lovell & Picklers, 1972). Greenberg, Kadowitz, Diecke & Long (1973) observed that superfused mesenteric arteries and veins showed greater contractile responses to noradrenaline (NA), $BaCl_2$ and KCl in the presence of PGE_1 and PGE_2. Prostaglandins have also been shown to enhance angiotensin and 5-hydroxytryptamine-induced contractions in artery and vein preparations from cats and dogs (Khairallah, Page & Turker, 1967; Greenberg *et al.*, 1973). Recently Couper & McLennan, (1978) showed that in the rat mesenteric artery preparation, indomethacin depressed responses to NA. The responses were restored by prostaglandins E_2, E_1, A_1, $F_{2\alpha}$, and A_2 in descending order of potency. However, these authors noted that at concentrations equi-effective in restoring depressed responses to control levels, PGA_1 but not PGE_2 potentiated NA-induced vasoconstrictor responses. On the other hand, Malik, Ryan & McGiff (1976) observed that PGE_2 potentiated NA vasoconstriction in rat and rabbit mesenteric arteries.

We have also consistently observed that PGE_2 potentiates NA-induced vasoconstriction in the rat mesenteric artery preparation. The present studies were undertaken to analyse the mechanism of this potentiation.

Methods

Preparation of mesenteric artery for perfusion

Surgical operations were carried out according to the method of McGregor (1965). Adult male rats weighing 250 g and above were anaesthetized with diethyl ether or chloroform. The abdomen was opened and the pancreatic-duodenal, ileo-colonic branches of the superior mesenteric artery were all tied off. The dorsal aorta was ligated a few mm anteriorly and posteriorly from its junction with the superior mesenteric artery. The latter was then isolated by cutting round the intestinal borders of the mesentery. The artery was cannulated and perfused with Krebs solution of the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25

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and glucose 11.5. The solution was bubbled with a mixture of 5% CO_2 and 95% O_2 and was perfused through the tissue at a constant flow rate of 4 ml/min with a Watson-Marlow constant flow inducer (type MHRE-88). The preparation was carefully arranged on blotting paper (moistened with Krebs solution) which was placed on the surface of a 250 ml conical flask in which water at 37°C circulated. The preparation was lightly covered with moist cotton wool which was periodically wetted with warm Krebs solution from a pipette. A lamp placed above the flask ensured that the whole preparation was maintained at 37°C throughout the experiment. Changes in perfusion pressure were recorded by a Bell & Howard pressure transducer (type 4-327-L-223) connected to one arm of the arterial cannula. The output of the transducer was displayed on a Devices M.19 recorder. Drugs were injected through a pressure tubing placed just before the rollers on the inlet side of the constant flow inducer in volumes not exceeding 0.2 ml at 5 min intervals. When the artery was perfused with prostaglandins or antagonist, the desired concentration of the drug was included in the Krebs solution. In all cases, the arteries were allowed to equilibrate for at least 30 min before the start of an experiment.

Inactivation of prostaglandins on perfusion through the rat mesenteric artery

Different concentrations of PGE_2 and $PGF_{2\alpha}$ were perfused through the rat mesenteric artery. The effluent was collected and assayed on the rat stomach strip (Vane, 1957) or rat colon (Regoli & Vane, 1964) against the parent prostaglandin solutions. The assay tissues were superfused with Krebs solution containing atropine, mepyramine, methysergide (each 100 ng/ml), propranolol and phentolamine (each 200 ng/ml). Percentage inactivation was calculated from the difference between the concentration of prostaglandins in the effluent and concentration in the parent solution.

Inactivation of prostaglandins in the pulmonary vascular bed of the guinea-pig lung

Guinea-pigs of either sex weighing between 300 and 500 g were killed by neck fracture and exsanguinated. The lungs were isolated and set up for perfusion through the pulmonary artery as described by Okpako (1971). When the effluent was free of prostaglandin-like activity, different concentrations of PGE_2 and $PGF_{2\alpha}$ were perfused through the lungs. In the concentrations used (0.01 and 0.1 μg/ml), the effluent showed no detectable activity on the rat stomach strip or rat colon suggesting that there was near complete inactivation of the prostaglandins on passage through the pulmonary vasculature. The effluent, presumed to contain metabolites inactive on the assay tissues, was used to study a possible effect of metabolites on noradrenaline-induced vasoconstriction in the rat mesenteric artery.

Reserpine-treatment of rats

Rats were injected with reserpine (10 mg/kg i.p.) daily for three days before the mesenteries were dissected out and prepared as described above.

Statistical analysis

Results are expressed as mean ± standard error of the mean (s.e. mean), where (n) represents the number of observations in the group. The significance of difference between the grouped data was evaluated by Student's t-test where appropriate. $P = 0.05$ was taken as significant.

Drugs

The following drugs were used: (−)-noradrenaline (Sigma), prostaglandins E_2 and $F_{2\alpha}$ (UpJohn & Co.), phentolamine mesylate (Rogitine Ciba), methoxamine hydrochloride (Wellcome), reserpine (Ciba). Stock solutions of PGE_2 and $PGF_{2\alpha}$ were made in 95% ethanol while that of noradrenaline was in 0.1 N HCl. All stock solutions were stored at −20°C, and diluted just before use.

Results

Vasoconstrictor responses of the rat mesenteric artery to noradrenaline and potassium

Effective doses of NA caused an increase in perfusion pressure which was taken to indicate arterial vasoconstriction. As shown by Macgregor (1965), the vasoconstrictor effect of NA was clearly dose-dependent and was rapidly reversible. The threshold dose of NA was about 0.1 μg injected into the perfusion fluid. The sensitivity range we used was such that the absolute maximum pressure output of the arteries could not be recorded. The highest recordable response in each experiment (achieved with about 1.6 μg NA) was regarded as the 'apparent maximal response' when calculating potentiation factors (see below). When first set up, responses to a given dose of NA tended to increase with time. It was therefore necessary to inject NA repeatedly until the response had become stable. This required a period of 30 to 45 min. Thereafter, dose-response curves were constructed to NA. In each experiment, at least three responses were recorded for each dose of NA before the introduction of a modifying drug. Potassium

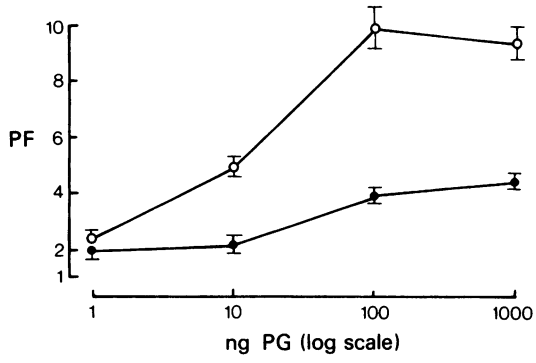

Figure 1 Prostaglandin-induced potentiation of noradrenaline vasoconstrictor responses in relation to prostaglandin dose in rat mesenteric artery. (○) Prostaglandin E_2 (PGE_2); (●) $PGF_{2\alpha}$. Each point is a mean of measurements from 6 separate preparations. Vertical bars are s.e. mean.

chloride also caused dose-dependent rapidly reversible vasoconstriction with a threshold of about 0.5 mg.

Interaction of prostaglandins with noradrenaline, methoxamine and potassium

In concentrations that did not show any direct vasoconstrictor or dilator effects, PGE_2 and $PGF_{2\alpha}$ greatly potentiated responses to NA. The enhancement of NA responses occurred within 90 s of introducing the prostaglandin and persisted for up to 45 min, after the prostaglandin was washed out. In order to obtain an estimate of the degree of enhancement, a potentiation factor (PF) was measured according to Trendelenburg, (1963). Dose-response curves to NA, methoxamine and potassium chloride were constructed before and in the presence of prostaglandins. NA and methoxamine dose-response curves were shifted in a parallel fashion to the left. The PF was the ratio:

$$\frac{\text{Dose of vasoconstrictor causing 50\% apparent maximal response before prostaglandin}}{\text{Dose of vasoconstrictor causing 50\% apparent maximal response in the presence of prostaglandin}}$$

The results are shown in Figure 1. It can be seen that the prostaglandin-induced potentiation was dose-dependent. PGE_2 was significantly more potent than $PGF_{2\alpha}$ in enhancing NA vasoconstrictor responses ($P < 0.005$) at all dose levels. Vasoconstrictor responses to potassium chloride were not enhanced by PGE_2 or $PGF_{2\alpha}$ (Figure 2). Methoxamine was also potentiated by PGE_2 and $PGF_{2\alpha}$ (Figure 3). The degree of potentiation was determined from methoxamine dose-response curves constructed in the absence and in the presence of the prostaglandins (10

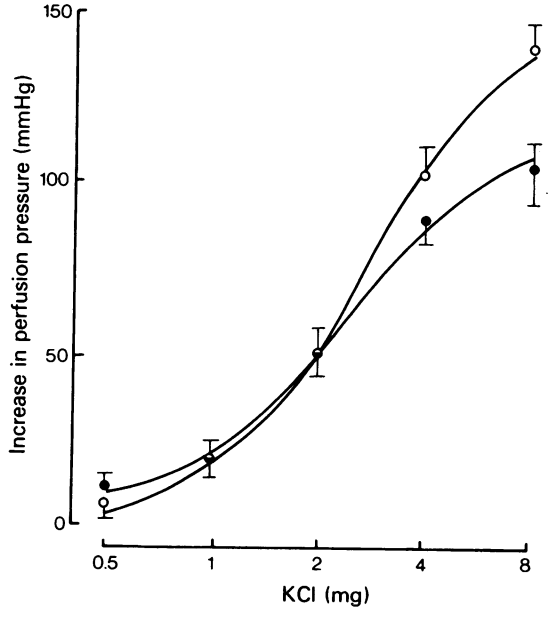

Figure 2 Effect of prostaglandin E_2 (PGE_2 10 ng/ml) on vasoconstrictor responses of rat mesenteric artery to potassium chloride: (○) control responses; (●) responses in the presence of PGE_2. Each point is a mean of measurements from at least 6 separate preparations. Vertical bars represent s.e. mean.

ng/ml). The PF values were 3.8 ± 0.3 ($n = 6$) for $PGF_{2\alpha}$ and 7.3 ± 0.4 ($n = 6$) for PGE_2.

Inactivation of prostaglandins in rat mesenteric arterial bed

The results summarized in Table 1 show that when 10 and 100 ng/ml PGE_2 and $PGF_{2\alpha}$ were perfused through the rat mesenteric artery, about 20% of each was inactivated, It was decided to inactivate the prostaglandins by passage through the guinea-pig lung pulmonary vasculature, and then to determine the effect of the perfusate effluent on the vasoconstrictor responses to NA. When 10 and 100 ng/ml PGE_2 and $PGF_{2\alpha}$ were perfused through guinea-pig lungs, the effluent showed no detectable activity in the assay tissues (rat stomach strip for PGE_2 and rat colon for $PGF_{2\alpha}$). The sensitivity of the assay tissues was good enough to detect 0.5 ng/ml. The effluent, used immediately after collection, did not enhance vasoconstrictor responses to NA (Table 2).

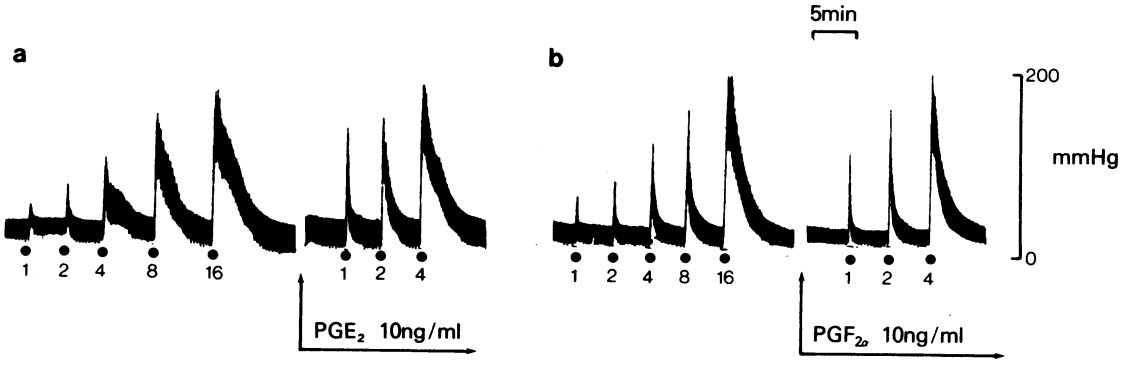

Figure 3 Potentiation of methoxamine-evoked vasoconstrictor responses by prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ in rat mesenteric artery. The first set of responses in each panel are control responses. PGE_2(a) or $PGF_{2\alpha}$ (b), in the concentration shown was introduced at the arrow. Methoxamine responses were repeated 5 min after adding a prostaglandin. The doses of methoxamine under the dots are in μg. (a) and (b) are from separate mesenteric artery preparations.

Effect of drugs affecting the adrenergic neurone

Reserpine In arteries from reserpine-treated rats PF for PGE_2 (10 ng/ml) was 9.6 ± 1.1 (n = 6). This value was significantly greater than the control value of 5.0 ± 0.9 (n = 6) (analysis of variance $P < 0.005$).

Bretylium In the presence of bretylium, (10 μg/ml), PF for PGE_2 was 3.6 ± 0.8 (n = 6). This value was significantly lower than the control (analysis of variance, $P < 0.005$).

Effect of external Ca^{2+} on noradrenaline potentiation by prostaglandin E_2

In one series of experiments, responses to NA were elicited in Krebs solution from which calcium chloride had been omitted (Ca^{2+}-free Krebs). After allowing the preparation to equilibrate in Ca^{2+}-free Krebs, 0.4 μg NA was injected repeatedly at 5 min intervals to determine the reproducibility of the NA responses in the absence of external calcium. Responses to NA were sustained during repeated application for 1 h. Thereafter, a gradual decline in the vasoconstrictor responses was evident. The mean responses to NA in Krebs containing 2.5 mM Ca^{2+} and those obtained within 1 h of Ca^{2+} removal were not significantly different at any dose used (Student's t test, $P > 0.05$).

The effect of PGE_2 on NA vasoconstriction in Ca^{2+}-free Krebs was studied by constructing dose-response curves to NA in the absence and presence of PGE_2 (Figure 4). Prostaglandin-induced potentiation of NA responses was absent in Ca^{2+}-free Krebs solution. The relationship between PGE_2-induced potentiation and the concentration of Ca^{2+} in the perfusion medium was examined by determining PF values for PGE_2 (10 ng/ml) in Krebs solution containing different concentrations of Ca^{2+}. A separate preparation was used at each Ca^{2+} ion concentration. The magnitude of potentiation (PF values) increased with increasing Ca^{2+} concentration up to 2.5 nM. A further

Table 1 Inactivation of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ in rat mesenteric artery

Concentration of PG in perfusion medium (ng/ml)	*Mean % inactivation of infused PG* ± s.e. mean PGE_2	$PGF_{2\alpha}$
10	20.6 ± 3.1 (8)	23.0 ± 3.9 (8)
100	19.0 ± 2.1 (6)	20.0 ± 2.5 (6)

The figures in parentheses represent number of experiments.

Table 2 Effect of two concentrations of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$, after passage through guinea-pig pulmonary circulation, on vasoconstrictor responses to noradrenaline in isolated mesenteric artery of rat

Concentration of PG used to perfuse the lungs (ng/ml)	*Potentiating effect of perfusate effluent* (PF ± s.e. mean) PGE_2	$PGF_{2\alpha}$
10	1.08 ± 0.08 (8)	1.14 ± 0.05 (8)
100	1.4 + 0.06 (6)	1.28 + 0.2 (8)

The figures in parentheses refer to the number of experiments from which the means were calculated.

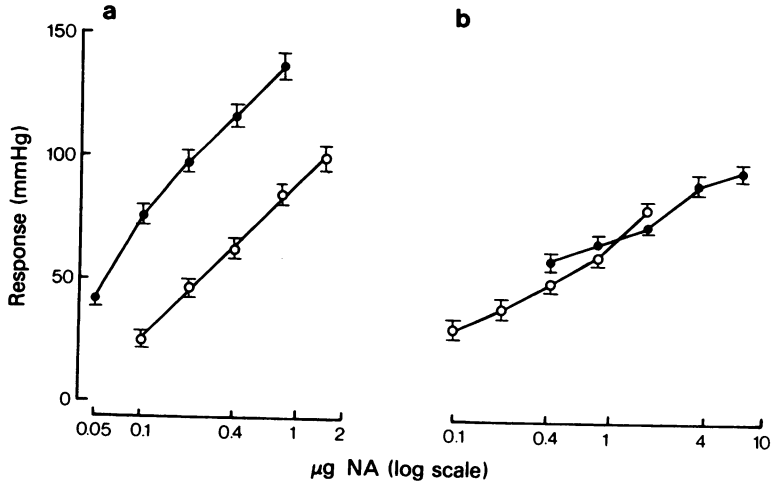

Figure 4 Effect of prostaglandin E_2 (PGE_2) (10 ng/ml) on noradrenaline vasoconstrictor responses of rat mesenteric artery in 2.5 mM calcium Krebs solution (a) and calcium-free Krebs (b). (○) Control responses; (●) responses in the presence of PGE_2. (a) and (b) are from separate preparations. Each point on the graph is the mean of measurements from 6 separate artery preparations. Vertical bars represent s.e. mean.

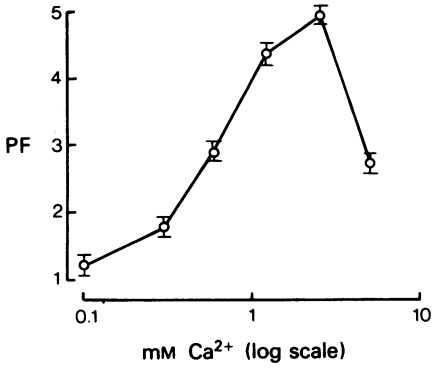

Figure 5 Effect of increasing calcium chloride concentration on noradrenaline vasoconstrictor potentiation (PF) caused by prostaglandin E_2 (PGE_2) (10 ng/ml) in rat mesenteric artery. Each point is a mean of measurements made in 7–8 preparations. Vertical bars represent s.e. mean.

increase in calcium concentration resulted in a decrease in the degree of potentiation (Figure 5).

Discussion

In the range of concentrations used PGE_2 and $PGF_{2\alpha}$ had no measurable effects on the isolated mesenteric arteries of the rat but greatly enhanced the vasoconstrictor action of NA. This result is in agreement with results obtained in other vascular smooth muscle preparations (see Introduction), but different from that of Couper & Mclennan (1978). The discrepancy may be due to the fact that these authors used higher doses of NA and PGE_2 than those used in the present experiments. In our experiments, the degree of potentiation produced by the highest concentration of PGE_2 (1000 ng/ml) was less than that produced by 100 ng/ml (Figure 1).

The results suggest that products of PGE_2 or $PGF_{2\alpha}$ metabolism did not contribute to enhancement of NA vasoconstriction since the prostaglandins after perfusion through guinea-pig lung did not show a potentiating effect. PGE_2 and $PGF_{2\alpha}$ undergo more than 90% inactivation on single passage through the guinea-pig pulmonary vasculature (Piper, Vane & Wyllie, 1970; Crutchley & Piper, 1975).

Both PGE_2 and $PGF_{2\alpha}$ potentiated the effects of NA after the adrenergic neurones had been depleted of NA by prior reserpine treatment or blocked with bretylium. The potentiation could not therefore be related to endogenous release of NA from adrenergic nerve endings. Potentiation of NA responses was significantly greater in arteries from reserpine-treated rats than in control arteries, while bretylium reduced the degree of potentiation. The mechanism by which reserpine and bretylium quantitatively affected prostaglandin enhancement of NA vasoconstriction is not yet known, but an additive effect with reserpine might be expected if PGE_2 was acting in a similar way to reserpine. Reserpine-induced supersensitivity has been attributed to an enhanced ability of the reserpine-treated tissue to retain and utilise Ca^{2+} (Carrier & Hester, 1976). As pointed out below,

prostaglandin enhancement of NA action seems also to be related to Ca^{2+} fluxes.

Prostaglandin enhancement of NA vasoconstriction does not seem to involve inhibition of neuronal uptake of NA since methoxamine responses were also enhanced by PGE_2 and $PGF_{2\alpha}$. Methoxamine is not a substrate for the uptake$_1$ mechanism. Its vasoconstrictor action is not potentiated by cocaine (Trendelenburg, Maxwell & Pluchino, 1970; see also Couper & Mclennan 1978).

NA vasoconstriction was maintained in Ca^{2+}-free Krebs. Since it is known that vascular smooth muscle contraction requires Ca^{2+} (Burks, Whitacre & Long, 1967) it would appear that in this preparation, the Ca^{2+} required for contraction, originated from an intracellular source. Hinkle (1965) and Hudgins & Weiss (1968) have also attributed the persistence of NA vasoconstrictor effects in the absence of external Ca^{2+} to utilisation of sequestered calcium. Our finding that the prostaglandin enhancement of NA vasoconstriction was absent in Ca^{2+}-free Krebs but increased in proportion to the concentration of Ca^{2+} ions in the external medium suggested strongly that the prostaglandin-induced enhancement was due to increased influx of Ca^{2+} from the external medium during NA stimulation.

Casteels & Droogmans (1976) and Kitamura, Kuriyama & Suzuki (1976) have shown that low concentrations of NA can cause contractions in rabbit ear and pulmonary arteries without depolarization of the smooth muscle cells. Since it is generally accepted that the resting plasma membrane is impermeable to calcium, it can be concluded that such NA vasoconstriction involved mobilisation of intracellular calcium without depolarization of smooth muscle cells, that is, a pharmacomechanical coupling (Somlyo & Somlyo, 1968a). Such a mechanism would explain why in the present experiments, NA-induced constrictor responses obtained in the presence and absence of external calcium were not significantly different in the short run. PGE_2 in low doses can cause depolarization of smooth muscle cells (Suzuki, Osa & Kuriyama, 1976) and increase membrane permeability (Horton, 1963; Crunkhorn & Willis, 1971; Thomas & West 1974). The mechanism of PGE_2 potentiation of NA vasoconstriction may be explained as follows: NA in the presence of PGE_2 activates the pharmacomechanical pathway, but in addition, NA vasoconstriction involves calcium from the external medium, either because PGE_2 increases membrane permeability to calcium or because PGE_2 enables NA to cause depolarization. It is known that membrane permeability to calcium increases as the membrane becomes depolarized. On the other hand, potassium which activates the electromechanical pathway (Somlyo & Somlyo 1968a) by depolarizing the cell membrane and facilitating influx of external calcium (Hudgins & Weiss (1968) is not enhanced by PGE_2 or $PGF_{2\alpha}$. This mechanism is similar to that proposed for the potentiation of NA vasoconstriction by tetraethylammonium in rabbit aorta (Kalsner 1973) and aminopyridines (Glover, 1978) in rabbit isolated ear artery. The proposed mechanism also explains why bretylium, which is known to possess local anaesthetic properties, reduced the potentiation of NA vasoconstriction by PGE_2.

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