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RESTORATION OF VASOCONSTRICTOR RESPONSES TO NORADRENALINE BY PROSTAGLANDIN E_2 AFTER α-ADRENOCEPTOR BLOCKADE IN RAT ISOLATED MESENTERIC ARTERY

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1 The effects of prostaglandin E_2 (PGE_2) on responses to noradrenaline (NA) after α-adrenoceptor blockade were studied in the isolated mesenteric artery of the rat.

2 Phentolamine (32 nM) tolazoline (41 μM) and yohimbine (1.28 μM) blocked NA-induced vasoconstriction competitively with dose-ratios of 13.9 ± 1, 22.0 ± 1 and 26.6 ± 0.9 respectively.

3 PGE_2 (28 nM) restored responses to NA during α-adrenoceptor blockade and reduced NA dose-ratios to 2.8 ± 0.1 (phentolamine), 5.9 ± 0.4 (tolazoline) and 1.7 ± 0.1 (yohimbine).

4 At low concentrations (0.29 nM), phenoxybenzamine blockade of NA-induced vasoconstriction was also antagonized by PGE_2.

5 PGE_2 did not reduce the pA_2 of the competitive antagonists; therefore the antagonism of α-adrenoceptor block by PGE_2 was not due to a reduction in the affinity of the antagonist for the receptor.

6 The calcium ionophore, A23187, also antagonized competitive α-adrenoceptor blockade but was less potent than PGE_2.

7 Evidence is provided to suggest that although both PGE_2 and A23187 can potentiate the action of NA in this preparation, the two compounds probably reverse α-adrenoceptor blockade by different mechanisms.

8 Inhibition of NA-induced vasoconstriction caused by the calcium antagonists cinnarizine, verapamil and high concentrations of phenoxybenzamine (>2 nM) were not affected by PGE_2.

9 It is proposed that PGE_2 restores responses to NA after α-adrenoceptor blockade by increasing intracellular Ca^{2+} ion concentration or by activating α-adrenoceptor-associated Ca^{2+} channels.

Introduction

Since the finding (Vane, 1971) that aspirin-like drugs inhibit prostaglandin synthesis, this group of compounds have been used to study the probable role of prostaglandins in physiological functions. Examples of this kind of investigation are those in the rabbit jejunum (Ferreira, Herman & Vane, 1976) and guinea-pig ileum and colon (Bennett, Eley & Stockley, 1975a, b; 1976), in which prostaglandin sythetase inhibitors reduce muscle tone. This effect can be reversed by low concentrations of prostaglandins. It is therefore presumed that muscle tone is maintained by endogenous prostaglandin generation. In the rat mesenteric artery preparation, Horrobin, Manku, Karmali, Nassar & Davies (1974) showed that aspirin and indomethacin inhibited noradrenaline-induced vasoconstriction. The inhibitory action of the prostaglandin synthetase inhibitors was reversed by low concentrations of prostaglandin E_2 (PGE_2). The authors therefore also concluded that the action of noradrenaline involved endogenous prostaglandin generation. This conclusion involves the assumption that the inhibitory action of the prostaglandin synthetase inhibitors is due entirely to prevention of endogenous formation of prostaglandins. Horrobin *et al.* (1974) thus argue that noradrenaline vasoconstriction in the rat mesenteric artery is mediated by prostaglandins.

In the course of studies with α-adrenoceptor antagonists in the rat isolated mesenteric artery, we have observed that the effects of phentolamine, tolazoline and yohimbine in blocking noradrenaline-induced vasoconstriction, can also be reversed by low concentrations of PGE_2. Since these antagonists are not known to inhibit prostaglandin synthesis, it was of interest to investigate the mechanism by which PGE_2 restored responses to noradrenaline during α-adrenoceptor blockade. A preliminary account of these findings has been published (Adeagbo, 1980).

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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 pancreatico-duodenal and 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 juction with the superior mesenteric artery. The latter was then isolated by cutting round the intestinal borders of the mesentery. Thereafter the whole preparation was quickly transferred on to a glass surface maintained at 37°C. The artery was cannulated and perfused at the rate of 4 ml/min 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 and glucose 11.5. The solution was bubbled with 5% CO_2 and 95% O_2 and perfused 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 moistened with warm Krebs solution from a pipette. An Angle-poise lamp arranged above the flask ensured that the whole preparation was maintained at 37°C throughout the experiment. Changes in perfusion pressure were recorded on a Bell & Howell pressure transducer (Type 4-327-223) on a Devices M. 19 recorder. Drugs were injected through pressure tubing placed just before the rollers of the constant flow inducer in volumes not exceeding 0.2 ml at 5 min intervals. In cases where the artery was perfused with prostaglandins or antagonists, the Krebs solution contained the required concentration of the drug. In all cases, the arteries were allowed to equilibrate for at least 30 min before the start of the experiment. Ca^{2+}-free Krebs was prepared by omitting calcium chloride from the constituents of normal Krebs solution (Bucks, Whitacre & Long, 1967).

Determination of the degree of antagonism

Dose-ratios (DR) for competitive antagonists against noradrenaline (NA) were measured in the presence of at least three concentrations of antagonist. pA_2 values were determined from A-S plots (Arunlakshana & Schild, 1959).

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 grouped data was evaluated by Students t test where appropriate. $P<0.05$ was taken as significant.

Drugs used

(−)-Noradrenaline free base (Sigma Chem Co), prostaglandins E_2 and F_2 (Upjohn Ltd), phentolamine HCl (Ciba), phenoxybenzamine HCl (Pbz) (Smith, Kline & French Ltd) cinnarizine (Janssen Pharmaceuticals Ltd), verapamil HCl (Cordilox ampoules, Abbot Laboratories Ltd), and A23187 (Elli-Lilly) were used. Stock solutions of PGE_2 and $PGF_{2\alpha}$ were prepared in 96% ethanol, cinnarizine was dissolved in 50% methanol and phenoxybenzamine in acidified ethanol (Benfey & Grillo, 1963). Noradrenaline stock solution was made up in 0.1N HCl. All stock solutions were stored at −20°C and diluted freshly with normal saline or Krebs solution just before use.

Results

α-Adrenoceptor blockade and its reversal by prostaglandin E_2

Phentolamine (32 nM–2.52 μM), tolazoline (2.5 μM–40 μM) and yohimbine (50 nM–1.28 μM) blocked NA in a competitive manner: (a) the NA dose-response curve in the presence of the antagonist was shifted to the right and parallel to the control curve, and (b) the slopes of the A-S plots, 0.96 ± 0.04 (phentolamine), 1.03 ± 0.04 (tolazoline) and 0.85 ± 0.02 (yohimbine) ($n=8$ in each case) were not significantly different from 1 ($P>0.05$).

Results with phenoxybenzamine (Pbz) were more complicated. In low concentrations (< 2nM), the blockade of NA was surmountable by increasing the dose of NA. If NA dose-response curves were repeated after a brief contact with Pbz (less than 30 min), the block showed characteristics of competitive antagonism. For example, when pA_2 was calculated from the equation: $pA_2 = \log (DR - 1) - \log (I)$, where (I) = concentration of Pbz and DR = dose-ratio, the values obtained with 0.074 nM, 0.29 nM and 1.16 nM Pbz were 10.58 ± 0.5, 10.65 ± 0.7 and 10.55 ± 0.9 respectively ($n=6$ in each case). The values were not significantly different from one another ($P>0.05$). This is indicative of competitive antagonism (Mackay, 1978). With a given concentration of Pbz, the degree of block increased with prolongation of the time of contact with Pbz and the block exhibited characteristics of non-competitive antagonism (see Figure 2). The block of

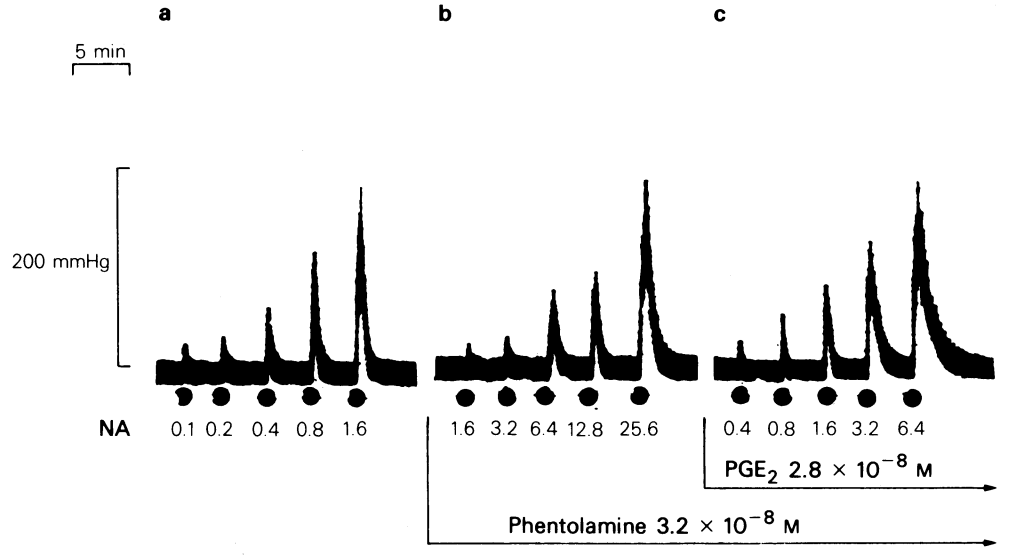

Figure 1 Restoration by prostaglandin E_2 (PGE_2) of responses to noradrenaline (NA) in isolated mesenteric artery of the rat during α-adrenoceptor blockade by phentolamine. The doses below the tracing are μg NA injected into the perfusate. (a) Responses to NA in normal Krebs solution; (b) responses to NA in the presence of phentolamine 3.2×10^{-8} M; (c) responses to NA in the presence of phentolamine and PGE_2 (2.8×10^{-8} M).

NA with concentrations of Pbz much higher than 2 nM was noncompetitive.

Antagonism of NA-induced vasoconstriction caused by phentolamine, tolazoline, and yohimbine were reversed by PGE_2 (Figure 1), the degree of reversal being directly proportional to the concentration of the prostaglandin. Antagonism of NA-induced vasoconstriction by Pbz in the concentration range 0.074 nM – 0.29 nM, was also reversed by PGE_2 (Figure 2). Blockade of NA responses increased with increase in Pbz contact time. Therefore it should be noted that NA block would be greater in Figure 2c

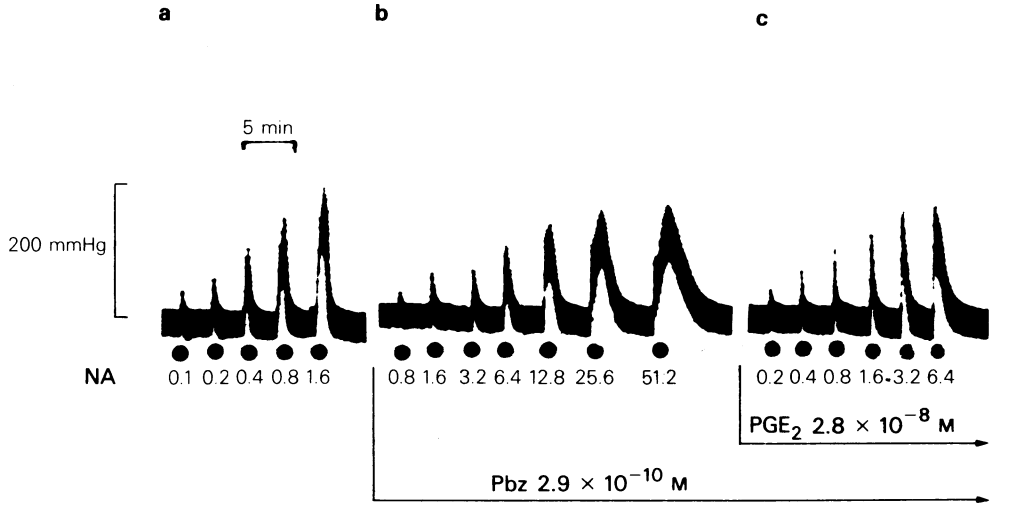

Figure 2 Restoration by prostaglandin E_2 (PGE_2) of responses to noradrenaline (NA) in isolated mesenteric artery of the rat during α-adrenoceptor blockade by phenoxybenzamine 0.29 nM. The doses below the tracing are μg NA injected into the perfusate. (a) Control responses to NA; (b) responses to NA in the presence of phenoxybenzamine (Pbz); (c) responses to NA in the presence of both Pbz and PGE_2. All responses were obtained from the same preparation.

Table 1 Effect of prostaglandin E_2 (PGE_2, 2.8×10^{-8} M) on noradrenaline (NA) blockade caused by various adrenoceptor antagonists in isolated perfused mesenteric artery of the rat

Antagonist	*Concentration of antagonist* (M)	*NA dose-ratio in the presence of antagonist alone* (DR_A)	*NA dose-ratio in the presence of antagonist + PGE_2* (DR_{APG})	*$DR_A - DR_{APG}$ (Reversal factor)*	*NA dose-ratio in the presence of PGE_2 *(Potentiation factor)*
Phentolamine	3.2×10^{-8}	13.9±1.0	2.5±0.1	11.4±0.8	
Tolazoline	4.1×10^{-5}	22.0±1.0	5.9±0.4	16.1±0.9	
Yohimbine	1.28 ± 10^{-6}	26.6±0.9	1.7±0.1	24.9±0.5	5.0±0.2
Phenoxybenzamine	2.9×10^{-10}	17.9±1.0	8.4±0.8	9.5±0.7	

The mean values were derived from 6–9 experiments.
*See text under Discussion.

than Figure 2b if PGE_2 were not present in 2c. Hence the extent of restoration of NA responses by PGE_2 is greater than is apparent from the trace. Such a situation may well account for the relatively smaller reversal factor for Pbz than the other α-adrenoceptor antagonist shown in Table 1. Blockade by Pbz at concentrations higher than 2nM was not reversed by PGE_2.

The extent of reversal by PGE_2 of the blockade by each of the antagonists was estimated by determining dose-ratios with the antagonist before and in the presence of different concentrations of PGE_2. Concentrations of PGE_2 ranging from 2.8×10^{-9} M to 2.8×10^{-7} M dose-dependently reduced the NA dose-ratios obtained with the four antagonists (Table 1). For a given antagonist, the effect of PGE_2 on the NA dose-ratio was dependent on the dose of PGE_2 (Figure 3).

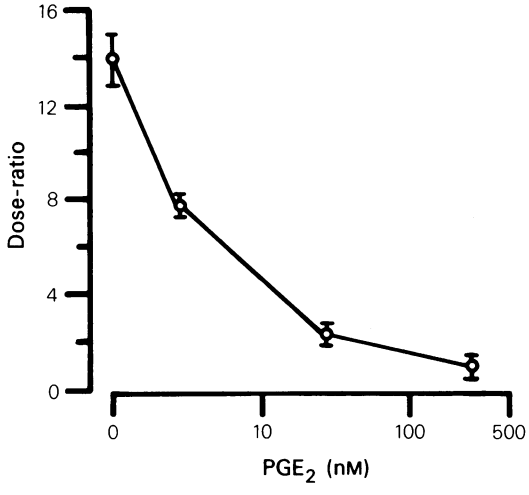

Figure 3 Effect of increasing concentrations of prostaglandin E_2 (PGE_2) on dose-ratios obtained with noradrenaline in the presence of phentolamine 32 nM in isolated mesenteric artery of the rat. Each point is a mean of 6 measurements; vertical lines show s.e.mean.

The ability of PGE_2 to reverse the competitive blockade of α-adrenoceptors might suggest that PGE_2 interferes with antagonist-receptor interaction. If this were so, different binding characteristics for the antagonist might be expected. To test this possibility, the pA_2 for each competitive antagonist was determined against NA before and in the presence of 2.8×10^{-8} M PGE_2. The results, presented in Table 2 show that PGE_2 did not reduce the apparent affinity of the antagonist for the receptor. In fact, PGE_2 significantly increased the pA_2 values for phentolamine and tolazoline.

Effect of prostaglandin E_2 on blockade of noradrenaline by cinnarizine and verapamil

Both cinnarizine and verapamil are potent antagonists of the excitation-contraction coupling system in vascular smooth muscle (Godfraind & Kaba, 1972). Neither of these two substances possesses specific α-adrenoceptor blocking activity. It was thus of interest to study the interaction of PGE_2 with these antagonists. Both cinnarizine and verapamil were potent antagonists of NA-induced vasoconstrictor responses. The block was non-competitive in nature and PGE_2 did not interfere with the blockade (Figure 4). KCl-induced responses were also inhibited by cinnarizine and verapamil and PGE_2 had no effect on their action.

Influence of Ca^{2+} ionophore (A23187) on α-adrenoceptor blockade by phentolamine, tolazoline and yohimbine

In a previous paper (Adeagbo & Okpako, 1980) evidence was presented to support the view that PGE_2 potentiated NA-induced vasoconstriction by facilitating Ca^{2+} influx. In order to determine whether the reversal of α-adrenoceptor blockade observed in the present experiments could also be explained on the same basis, we studied the interaction of the Ca^{2+} ionophore, A23187, with α-

Table 2 Effect of prostaglandin E_2 (PGE_2) on α-adrenoceptor antagonism in isolated mesenteric artery of the rat

	Normal Krebs		*During perfusion with PGE_2 2.8×10^{-8} M*	
Antagonist	*pA_2*	*Slope of A-S line*	*pA_2*	*Slope of A-S line*
Phentolamine	8.58 ± 0.11 ($n = 8$)	0.96 ± 0.04	8.95 ± 0.14* ($n = 8$)	0.92 ± 0.02^{NS}
Tolazoline	5.69 ± 0.01 ($n = 8$)	1.03 ± 0.04	6.15 ± 0.01* ($n = 8$)	0.97 ± 0.03^{NS}
Yohimbine	7.48 ± 0.07 ($n = 8$)	0.85 ± 0.02	7.54 ± 0.04^{NS} ($n = 8$)	0.83 ± 0.01^{NS}
Phenoxybenzamine	10.84 ± 0.01 ($n = 6$)	0.87 ± 0.01	10.55 ± 1.10^{NS} ($n = 6$)	0.83 ± 0.3^{NS}

*Significantly higher than controls ($P<0.005$); ^{NS} = Not significantly different from controls ($P>0.05$).

adrenoceptor antagonists. This compound facilitates excitation-contraction coupling by promoting Ca^{2+} influx across cell membranes (Reed & Lardy, 1972; Pressman, 1976). Like PGE_2, A23187 potentiated NA vasoconstriction over a wide range of doses. In a concentration of 1.9×10^{-6} M, A23187 potentiated NA almost nine fold. The effect of this concentration on α-adrenoceptor blockade by phentolamine, tolazoline and yohimbine was investigated. The results are shown in Table 3. It can be seen that A23187 considerably reduced NA dose-ratios obtained in the presence of the three antagonists.

Effect of Ca^{2+} lack

In one series of experiments, the interaction of NA with α-adrenoceptor antagonists and the influence of PGE_2 thereon were studied in Ca^{2+}-free Krebs. The results (Table 4) show that PGE_2 also reversed phentolamine, tolazoline and yohimbine block in Ca^{2+}-free Krebs.

Discussion

These results show that PGE_2 can restore responses to NA during competitive blockade of α-adrenoceptors by phentolamine, tolazoline, and yohimbine. Reversal of blockade of NA-induced vasoconstriction was also observed when Pbz was used in low concentrations. However, PGE_2 had no effect when NA-induced vasoconstriction was inhibited by the calcium antagonists, cinnarizine or verapamil and high doses of Pbz.

Our results are comparable to those of Maxwell, Plummer, Polvalski, Schneider & Coombs (1959) who observed that cocaine reversed the blocking actions of surmountable antagonists, but not those of unsurmountable antagonists of NA. However, these authors associated the reversal with cocaine-induced supersensitivity and suggested that cocaine might cause changes in the configuration of α-adrenoceptors. Since PGE_2 also enhances the sensitivity of the rat mesenteric artery to NA (Adeagbo

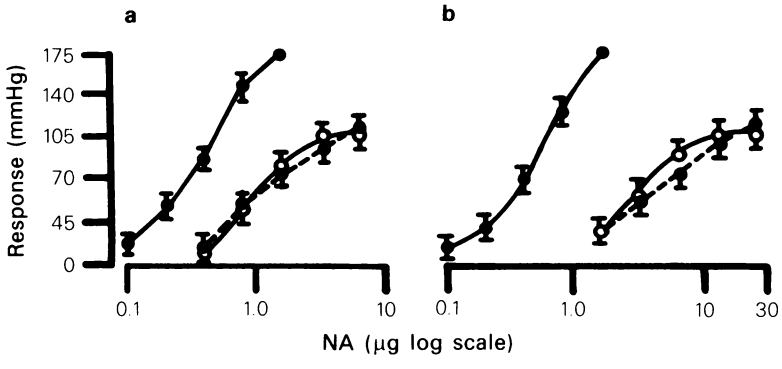

Figure 4 Failure of prostaglandin E_2 (PGE_2 28 nM) to restore responses to noradrenaline (NA) in isolated mesenteric artery of the rat in the presence of cinnarizine 1 μg/ml (a) or verapamil 0.5 μg/ml (b). (●——●) Control responses to noradrenaline; (○——○) responses to noradrenaline in the presence of cinnarizine (a) and verapamil (b); (●---●) responses to noradrenaline in the presence of antagonist plus PGE_2 28 nM.

Table 3 Effect of A23187 (1.6×10^{-6} M) on noradrenaline (NA) blockade caused by various α-adrenoceptor antagonists in isolated perfused mesenteric artery of the rat

Antagonist	*NA dose-ratio in the presence of antagonist alone* (DR_A)	*NA dose-ratio in the presence of antagonist + A23187* (DR_{A23187})	$DR_A - DR_{A23187}$ *(Reversal factor)*	*NA dose-ratio in the presence of A23187 alone (Potentiation factor)*
Phentolamine (3.2×10^{-8} M)	16.1 ± 1.3	6.7 ± 0.6	9.4 ± 1.1	
Tolazoline (4.1×10^{-5} M)	22.3 ± 1.0	14.3 ± 1.4	8.1 ± 0.9	8.7 ± 1.0
Yohimbine (1.28×10^{-6} M)	24.8 ± 0.4	17.0 ± 0.8	7.8 ± 0.5	

Each mean value is derived from six experiments. *See Discussion.

& Okpako, 1980) we have determined pA_2 values for various α-adrenoceptor antagonists with NA as agonist, before and in the presence of PGE_2. This approach was based on the principle that any alteration in the nature of the α-adrenoceptor might be expected to lead to changes in the binding characteristics of the receptor with its specific antagonist (Green & Fleming, 1967; Taylor & Green, 1971). The results showed that PGE_2 could not be said to restore NA responses in the presence of α-adrenoceptor blockade by altering the receptor in such a way as to reduce its affinity for the antagonist, since pA_2 values determined in the presence of PGE_2 were, if anything, increased. PGE_2 potentiates NA-induced vasoconstriction in this preparation by a mechanism that involves utilization of external Ca^{2+} (Adeagbo & Okpako, 1980). Part of the evidence for this view is that potentiation was absent in Ca^{2+}-free Krebs and increased in proportion to the concentration of Ca^{2+} in the external medium. Reversal of antagonism described here cannot be accounted for wholly in terms of enhancement of NA-induced vasoconstriction caused by PGE_2. Firstly, although NA potentiation is not observed in Ca^{2+}-free Krebs, antagonism of NA by phentolamine, tolazoline and yohimbine in Ca^{2+}-free Krebs was reversed by PGE_2 (see Table 4). This suggests that, unlike the potentiation phenomenon, external Ca^{2+} may not be involved in blockade reversal by PGE_2. Secondly, if enhancement of NA-induced vasoconstriction by PGE_2 could wholly account for the reversal, then the following relationship would be expected to hold for a given dose of PGE_2:

DR_P (Potentiation factor) = $DR_A - DR_{APG}$ (Reversal factor)

where DR_P = NA dose-ratio in the presence of PGE_2,
DR_A = NA dose-ratio in the presence of antagonist,
DR_{APG} = NA dose-ratio in the presence of antagonist + PGE_2.

For all the antagonists (Table 1) the reversal factors were significantly higher ($P<0.005$) than the potentiation factor obtained for NA with 2.8×10^{-8} M PGE_2.

In contrast, the partial reversal by A23187 of NA antagonism appeared to be due to its ability to enhance NA vasoconstriction, since A23187 reversed blockade of NA by the same order of magnitude as that by which it potentiated NA (Table 3). Furthermore A23187 did not restore responses to NA after blockade of α-adrenoceptors in Ca^{2+}-free Krebs. A23187 facilitates Ca^{2+} influx (Reed, 1968; Reed & Lardy, 1972; Pressman, 1976) which would account for potentiation of NA-induced vasoconstriction.

It is noteworthy that antagonism of NA by cinnarizine, Pbz (high dose) and verapamil was unaffected by PGE_2. All these agents can block

Table 4 Effect of prostaglandin E_2 (PGE_2, 2.8×10^{-8} M) on blockade of noradrenaline-induced effects in isolated mesenteric artery of the rat caused by various α-adrenoceptor antagonists in Ca^{2+}-free Krebs solution

Antagonist	*NA dose-ratio in the presence of antagonist alone* ($n = 6$)	*NA dose-ratio in the presence of antagonist + PGE_2* ($n = 6$)
Phentolamine (3.2×10^{-8} M)	17.6 ± 0.9	5.3 ± 0.6
Tolazoline (4.07×10^{-5} M)	27.2 ± 2.7	8.6 ± 0.8
Yohimbine (12.8 ± 10^{-7} M)	20.2 ± 1.0	1.9 ± 0.2

excitation-contraction (E-C) coupling. For instance, verapamil blocks E-C coupling in heart muscle by Ca^{2+} antagonism (Fleckenstein, Tritthart, Fleckenstein, Herbst & Grun, 1969) or specific block of potential-dependent Ca^{2+} permeability channels (Rasmussen & Goodman, 1977). Similarly, cinnarizine (Godfraind & Kaba, 1968; 1972) and Pbz in higher concentrations (Bevan, Osher & Su, 1963; Shibata & Carrier, 1967) have been shown to be Ca^{2+} antagonists. In contrast, none of the so-called competitive antagonists at the α-adrenoceptor site have been shown to be Ca^{2+} antagonists.

Moran, Swammy & Triggle (1970) proposed that activation of α-adrenoceptors by NA in vascular smooth muscle, causes a proportionate amount of bound intracellular Ca^{2+} ions to be released for contraction. α-Adrenoceptor blockade would therefore reduce the amount of bound Ca^{2+} mobilized by the agonist. The present finding that PGE_2 can restore agonist responses in the presence of α-adrenoceptor blockade without reducing the apparent affinity of the antagonist for the receptor suggests that the prostaglandin increases the availability of Ca^{2+} for contraction. Since restoration of NA responses can occur in normal as well as in Ca^{2+}-free Krebs, it is possible that the action of PGE_2 in this regard involves both facilitation of calcium influx (Eagling, Lovell & Piddes, 1972; Adeagbo & Okpako, 1980) and mobilization of bound intracellular Ca^{2+}. The finding that PGE_2 failed to reverse blockade caused by verapamil, cinnarizine and high concentrations of Pbz, is consistent with the view that calcium is required for the action of PGE_2.

So far there is no evidence that α-adrenoceptor blockade involves inhibition of prostaglandin synthesis. The α-adrenoceptor antagonists used in this study do not inhibit prostaglandin synthesis. The results thus demonstrate that PGE_2 may restore vasoconstrictor responses to NA even if the agent which reduced the responses did not inhibit endogenous generation of prostaglandins.

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