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THE INFLUENCE OF PROSTAGLANDINS ON NORADRENALINE-INDUCED VASOCONSTRICTION IN ISOLATED PERFUSED MESENTERIC BLOOD VESSELS OF THE RAT

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1 The report of the depression by indomethacin of vasoconstrictor responses to noradrenaline and their partial restoration by prostaglandin E_2 (PGE_2) and PGE_1 in rat isolated perfused mesenteric blood vessels was investigated. The further suggestion that prostaglandins may be necessary for the combination of noradrenaline with the α-adrenoceptor in this tissue was also studied.

2 The reported depression by indomethacin was confirmed and was further shown to be in the form of a concentration-dependent flattening of the noradrenaline concentration-effect curve.

3 A concentration-dependent restorative effect was observed for all prostaglandins studied. The decreasing order of potency for the restoration towards normal of the indomethacin-depressed responses to noradrenaline was: PGE_2, PGE_1, PGA_1, $PGF_{2\alpha}$, PGA_2.

4 The prostaglandins studied were not uniform in their restorative actions and could be separated into two groups. PGE_2 and PGE_1 restored responses towards the control level whereas PGA_1, PGA_2 and $PGF_{2\alpha}$ increased responses to an above control level and did so over a smaller concentration range. The possibility of several prostaglandin receptors is discussed.

5 At concentrations equi-effective in restoring depressed responses to control levels PGA_1 but not PGE_2, caused a parallel shift of the noradrenaline concentration-effect curve to the left and a small, gradual rise in the basal perfusion pressure.

6 The reason for the differing effects remains obscure but does not seem to involve a change in the α-adrenoceptor as indicated by the pA_2 of phentolamine. Furthermore, the restorative and potentiating effect of PGA_1 is not mediated by blockade of neuronal uptake of noradrenaline.

7 It appears that prostaglandins are required for the vasoconstrictor action of noradrenaline in rat mesenteric blood vessels and that this effect is distal to the drug-receptor interaction. The possible involvement of prostaglandins with intracellular calcium ions is discussed.

Introduction

Prostaglandins alter the reactivity of vascular smooth muscle by direct pressor and depressor actions (Nakano, 1973) as well as by modulating the release of noradrenaline from sympathetic nerve terminals (Hedqvist, 1970). Various authors have recently reported that prostaglandins also interact with vasoactive agents in a variety of tissues from a number of species. For example in some vascular tissues, prostaglandins of the E and A series have been shown to potentiate responses to a number of vasoactive agents (Greenberg & Long, 1973; Ercan & Turker, 1975; Armstrong, Blackwell, Flower, McGiff, Mullane & Vane, 1976; Malik, Ryan & McGiff, 1976) whereas inhibition has been observed in others (Kadar & Sunahara, 1969; Weiner & Kaley, 1969; Viguera & Sunahara, 1969; Kadowitz, 1972; Malik *et al.*, 1976). Generally the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), if studied, is opposite to PGE and PGA. There is unfortunately some disagreement as to the effects of prostaglandins when different investigators have studied the same vascular bed in the same species (Kadar & Sunahara, 1969; Greenberg & Long, 1973).

It is apparent from the many, sometimes conflicting reports that the interactions between prostaglandins and vasoactive agents are not only species- but also tissue-dependent. These indirect effects may be important physiologically as they generally occur at prosta-

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glandin concentrations well below threshold for their agonist actions.

Horrobin, Manku, Karmali, Nassar & Davies (1974) found that the vasoconstrictor response to a single dose level of noradrenaline in isolated perfused mesenteric blood vessels of the rat could be depressed and even abolished by the prostaglandin synthetase inhibitors aspirin and indomethacin, and partially restored by PGE_2 and PGE_1 but not $PGF_{2\alpha}$. These workers suggested that prostaglandins may play a physiological role in mediating the effects of vasoactive agents, and proposed that endogenous prostaglandin synthesis may be necessary for the combination of noradrenaline with its receptor, or for the chain of events occurring between receptor activation and smooth muscle contraction.

The aim of this study was to first confirm and then extend the findings of Horrobin *et al.* (1974). The effect of indomethacin on the entire noradrenaline concentration-effect curve has been investigated. A number of prostaglandins have also been examined for their ability to restore the indomethacin-depressed responses to noradrenaline with the aim of characterizing the prostaglandin receptors involved in this effect. Furthermore, the possible alteration of the nature of the α-adrenoceptor by prostaglandins or an action on noradrenaline uptake were also studied. Some of these results have been communicated to the Australasian Society of Clinical and Experimental Pharmacologists (McLennan & Coupar, 1976).

Methods

Male rats of the Sprague-Dawley strain were used, having a mean weight of 310 g (range 240–450 grams).

The isolated perfused mesentery preparation of the rat

The preparation was excised as described by McGregor (1965). Rats were anaesthetized with pentobarbitone sodium (40 mg/kg, i.p.). The artery was perfused from a reservoir with Krebs-Henseleit solution with added glucose, of the following composition (g/l):- NaCl 6.87, KCl 0.4, $MgSO_4.7H_2O$ 0.14, $NaH_2PO_4.2H_2O$ 0.18, $NaHCO_3$ 2.1, $CaCl_2$ 0.28 and D(+)-glucose 2.0. This solution was bubbled with 5% CO_2 in O_2 and was perfused through the tissue at a constant flow rate of 2 ml/min with a peristaltic roller pump (Cole-Palmer, Masterflex). The isolated perfused preparation floated on the surface of a 100 ml organ bath which was also filled with Krebs-Henseleit solution maintained at a temperature of 37°C and bubbled with a mixture of 5% CO_2 in O_2. The perfusion pressure was recorded via a side-arm of the arterial cannula with a Statham P23AC pressure transducer connected to a d.c. Grass polygraph. Another vertical but sealed side-arm acted as a bubble trap.

The perfusion solution was drawn from either of two reservoirs, one containing Krebs-Henseleit solution alone, another Krebs-Henseleit solution plus added noradrenaline. These reservoirs were connected to the pump by a tube each converging on a Y-piece, thus enabling perfusion with either solution by occluding the opposite tube. Vasoconstrictor responses were produced by perfusing the tissues with noradrenaline for 10 s at the constant rate of 2 ml/minute. In this manner, a known concentration of noradrenaline was delivered to the tissue. Infusion of noradrenaline produced a transient vasoconstriction which began within 20 s and reached a maximum within a further 5 to 10 seconds. Responses lasted about 1 min and were measured as changes in perfusion pressure (1 mmHg ≈ 133 Pa).

All other drugs were added to the perfusion reservoir, and the organ bath was flushed frequently throughout all experiments.

Experimental procedure

Experiments were preceded by an equilibration period of 60 to 90 minutes. Noradrenaline solutions were infused at 2 min intervals and any one tissue was exposed to only one prostaglandin type.

The effect of indomethacin on the noradrenaline concentration-effect curve

Responses were produced to noradrenaline, first in the absence, then in the presence of increasing concentrations of indomethacin (10, 25 and 62.5 μg/ml. The preparation was perfused with each indomethacin concentration for 30 min before the noradrenaline concentration-effect curve was obtained.

The effect of prostaglandins in the presence of indomethacin

Two similar responses (within 5 mmHg) were obtained to a concentration of noradrenaline (3.0 μg/ml) that elicited approximately 50% of the maximal response (EC_{50}), first in the absence, then after 15 min equilibration with indomethacin (25 μg/ml). This concentration was shown in the previous experiments to cause 50 to 70% depression of responses to noradrenaline. While maintaining indomethacin in the perfusate, prostaglandins were perfused through the tissue in progressively increasing concentrations, and responses to noradrenaline were obtained at each level. Concentration-effect curves were constructed of the ability of the various prostaglandins to restore the indomethacin-depressed responses to noradrenaline. Each prostaglandin concentration was main-

tained in the perfusate for 10 min before responses to noradrenaline were obtained.

The effect of prostaglandins on the normal noradrenaline concentration-effect curve

Responses were produced to noradrenaline, first in the absence, then with either PGE_2 or PGA_1. The prostaglandins were perfused through the tissue for 20 min before responses to noradrenaline were obtained. The concentrations used (PGE_2, 0.2 μg/ml and PGA_1, 2.0 μg/ml) were those observed to restore the indomethacin-depressed responses to noradrenaline to approximate control levels (Figure 2).

The effect of prostaglandins on the α-adrenoceptor

A series of four concentration-effect curves was constructed to noradrenaline, one in the absence and the three others in the presence of increasing concentrations of phentolamine. The EC_{50} was estimated from each curve and the pA_2 determined by the method of Arunlakshana & Schild (1959). This process was repeated in other tissues with either PGE_2 (0.2 μg/ml) or PGA_1 (2.0 μg/ml). These concentrations were once again chosen as being equi-effective in restoring the depressed responses to noradrenaline to approximate control levels.

Prostaglandins were perfused through the tissue for 20 min before control concentration-effect curves were obtained and each phentolamine concentration was equilibrated with the tissue for 15 min before the concentration-effect curve was constructed.

The effect of prostaglandin A_1 during uptake blockade

Responses were obtained to noradrenaline first in the absence and then in the presence of 5 and then 10 μg/ml of cocaine. The tissues were equilibrated with these concentrations for 10 min each. Separate tissues were then perfused with Krebs-Henseleit solution containing cocaine at 10 μg/ml throughout the experiments while the effect of PGA_1 was investigated on indomethacin-depressed responses to noradrenaline as described above.

In other tissues, responses to noradrenaline were produced first in the absence and then after 10 min equilibration with 17-β oestradiol.

Statistical analysis

A factorial experimental design was used for the investigation of the effects of indomethacin on responses to noradrenaline, and was subjected to an analysis of variance (Brownlee, 1949). Three factors were involved; preparations, concentrations of noradrenaline and concentrations of indomethacin. In subsequent experiments, a 6 point bioassay type of design was used in comparing pairs of curves for differences in regression and curvature, once again with an analysis of variance on the results (Colquhoun, 1971). Estimates of pA_2 values were compared with Dunnett's *t*-test for multiple comparisons (Dunnett, 1955). In all experiments statistical significance was taken to be when $P < 0.05$.

Drugs

Solutions of PGE_1, PGE_2, PGA_1 and PGA_2 were prepared at a concentration of 10 mg/ml in absolute ethanol. $PGF_{2\alpha}$ was made up at 10 mg/ml in distilled water. These were stored at −20°C and warmed to room temperature as required. Working stock solutions of the prostaglandins containing 1 mg/ml were prepared by further dilution with phosphate buffer 0.2 M (pH 6–7). Stock solutions of indomethacin were prepared at a concentration of 10 mg/ml in absolute ethanol. Final dilutions of all drugs were made in Krebs-Henseleit solution before use. In preliminary experiments, ethanol at the concentrations present in final prostaglandin or indomethacin dilutions was without effect on basal perfusion pressure or responses to noradrenaline. Ascorbic acid (0.3 μg/ml) which was without effect on basal perfusion pressure alone, was added to diluted noradrenaline as protection against oxidation. Final concentrations of all drugs are expressed in terms of the free acid or base. The following drugs were used:- cocaine hydrochloride B.P.; indomethacin (Merck, Sharp & Dohme, (Aust.) Pty. Ltd.); (−)-noradrenaline bitartrate (Sigma Chemical Co.); 17-β-oestradiol (Sigma Chemical Co.); pentobarbitone sodium (Sagital, May & Baker Ltd.); phentolamine mesylate (Regitine, Ciba-Geigy, Aust. Ltd.); prostaglandins A_1, A_2, E_1, E_2 and $F_{2\alpha}$ tromethamine salt (The Upjohn Company).

Results

As reported by other authors (McGregor, 1965; Malik & McGiff, 1974; Horrobin *et al.*, 1974), after an initial equilibration period, this preparation gave reproducible vasoconstrictor responses to noradrenaline and the baseline perfusion pressure remained steady over a period of several hours during perfusion with Krebs-Henseleit solution alone.

The effect of indomethacin on the noradrenaline concentration-effect curve

Infusion of noradrenaline through the tissue caused a transient, log concentration-related increase in perfusion pressure over a range of 0.5 to 32 μg/ml. This

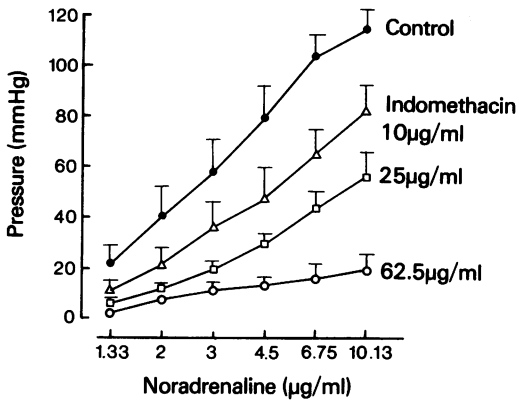

Figure 1 Effect of indomethacin on vasoconstrictor responses to noradrenaline. The ordinate scale in this and following figures represents the maximum increase in pressure produced in response to 10 s infusion of noradrenaline. Responses were elicited at 2 min intervals: (●) control; the other curves were obtained successively after 30 min perfusions with indomethacin; (△) 10 μg/ml, (□) 25 μg/ml and (○) 62.5 μg/ml. Each point on the curves is the mean from 7 tissues and the vertical bars represent the standard errors of the means (s.e. means) which are omitted when smaller than the symbol. The reduction in slope of the noradrenaline concentration-effect curve by indomethacin is concentration-related (analysis of variance, $P < 0.05$).

effect appeared to be linear from 1.33 to 10.13 μg/ml ($P < 0.05$).

Indomethacin in the perfusion fluid did not alter the basal perfusion pressure directly, but caused a flattening of the noradrenaline concentration-effect curve (Figure 1). This effect was rapidly reversible, with responses to noradrenaline returning to control levels within 10–20 min of reperfusing the tissue with Krebs-Henseleit solution alone. The control slope was significantly greater than the average slope obtained in the presence of indomethacin ($P < 0.05$). Furthermore, there was an indomethacin concentration-related trend in reduction of slope within the curves which was linear ($P < 0.05$).

The effect of prostaglandins in the presence of indomethacin

All five prostaglandins studied exhibited some degree of restorative action on indomethacin-depressed responses to noradrenaline (Figure 2) but had no effect on basal perfusion pressure in the presence of indomethacin. It was apparent that the prostaglandins could be divided into two classes. The first group, comprising PGE_1 and PGE_2, at concentrations up to 20 μg/ml restored responses toward but not above control levels whereas the second group, comprising

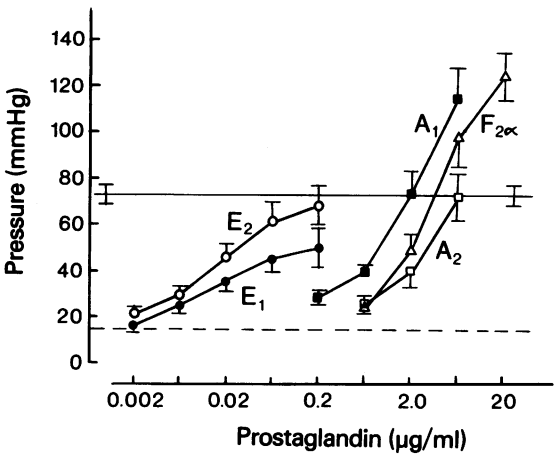

Figure 2 The effect of prostaglandins in restoring the indomethacin-depressed vasoconstrictor responses to noradrenaline. The upper, solid horizontal line represents the mean control responses to 10 s infusions of an approximate EC_{50} of noradrenaline (3.0 μg/ml) and the lower broken line represents the mean response to noradrenaline after 15 min equilibration of the tissue with indomethacin (25 μg/ml) in a total of 24 tissues. Mean responses to noradrenaline after perfusion with: (○) PGE_2, ($n = 7$); (●) PGE_1, ($n = 5$); (□) PGA_2, ($n = 4$); (■) PGA_1, ($n = 4$); (△) $PGF_{2\alpha}$ ($n = 4$), for 10 min at each concentration in the presence of indomethacin. Vertical bars indicate s.e. means. Lines E_1 and E_2 differ significantly in slope from the other lines (analysis of variance, $P < 0.05$).

PGA_1, PGA_2, and $PGF_{2\alpha}$, increased the responses to a greater than normal level and did so over a smaller range of concentrations. The slopes of the concentration-effect curves for the latter were significantly greater than for either PGE_1 or PGE_2 ($P < 0.05$).

The decreasing order of potency for restoring towards normal, depressed responses to noradrenaline was: PGE_2, PGE_1, PGA_1, $PGF_{2\alpha}$, PGA_2.

The effect of prostaglandins on the noradrenaline concentration-effect curve

The concentrations of PGE_2 (0.2 μg/ml) and PGA_1 (2.0 μg/ml) used were equi-effective in restoring indomethacin-depressed responses to noradrenaline to an approximate control level (Figure 2). PGE_2 at this concentration had no effect on the basal perfusion pressure but PGA_1 caused a small (<10 mmHg), gradual rise in the basal perfusion pressure. Neither PGE_2 nor PGA_1 significantly altered the slope of the control noradrenaline concentration-effect curve ($P > 0.05$). PGE_2 did not alter significantly the position of the control curve ($P > 0.05$) (Figure 3a) but

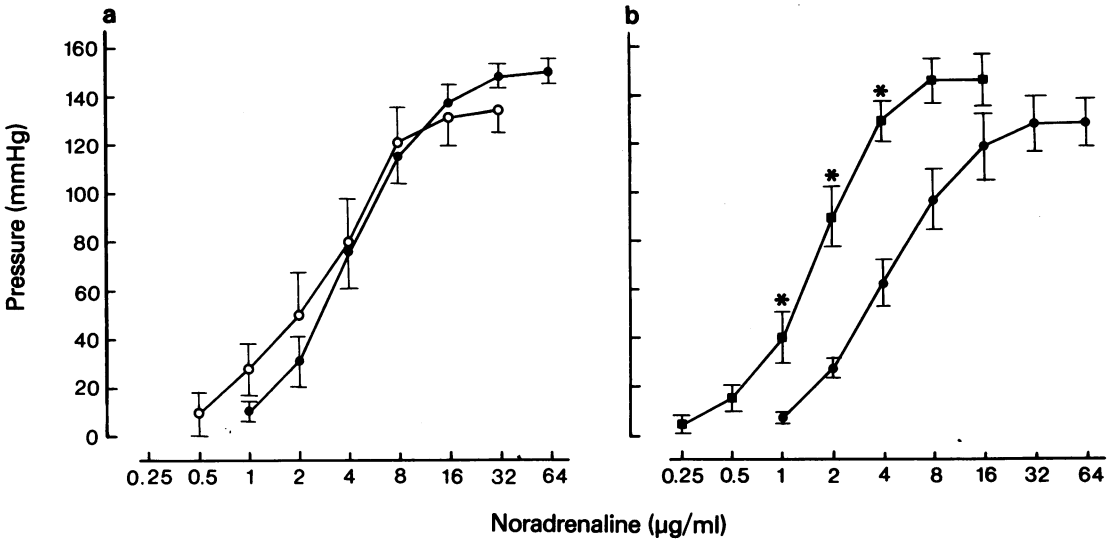

Figure 3 Comparison of equi-restorative concentrations of (a) prostaglandin E_2 (PGE_2) and (b) prostaglandin A_1 (PGA_1) on responses to noradrenaline. (a) (●) Control; (○) after perfusion of the tissue for 20 min with PGE_2 0.2 μg/ml. Each point on the curves represents the mean from 5 tissues and the vertical bars represent the s.e. means. Dose-ratio = 1.3 (95% confidence limits from 3.12 to 0.60). (b) (●) Control; (■) after perfusion of the tissue for 20 min with PGA_1 2.0 μg/ml.
*The line derived from the points is significantly different from the control concentration-effect curve (analysis of variance, $n = 7$). Dose-ratio = 4.6 (95% confidence limits from 9.25 to 2.60).

infusion of PGA_1 potentiated the effect of noradrenaline 4.6-fold (95% confidence limits from 9.25 to 2.60) (Figure 3b).

The effect of prostaglandins on the α-adrenoceptor

Phentolamine, in the range of concentrations from 10^{-9} M to 10^{-7} M, appeared to act as a competitive antagonist of noradrenaline in all experiments, causing a concentration-dependent parallel shift to the right of the agonists concentration-effect curve. Competitive antagonism (Arunlakshana & Schild, 1959) was also indicated by a slope of approximately −1 in a plot of $\log_{10}$ (dose-ratio −1) against the negative $\log_{10}$ of the molar concentration of phentolamine.

Perfusion of PGE_2 or PGA_1 through the tissue did not significantly alter the slope of the Arunlakshana-Schild plot and the mean slopes obtained from each treatment group did not differ significantly from −1 ($P > 0.05$).

The pA_2 as determined graphically from the intercept of the Arunlakshana-Schild plot was also not significantly altered by either PGE_2 or PGA_1 in the concentrations used ($P > 0.05$). The effects of equi-restorative concentrations of PGE_2 PGA_1 on the slope of the Arunlakshana & Schild plot and the pA_2 are presented in Table 1.

The effect of prostaglandin A_1 during uptake block

The possibility that the potentiating effect of PGA_1 on indomethacin-depressed responses to noradrena-

Table 1 The effect of prostaglandins A_1 (PGA_1) and E_2 (PGE_2) on the pA_2 of phentolamine for antagonism of noradrenaline

	n	*Slope (mean ± s.e.m.)*	*pA_2 (mean ± s.e.m.)*
Control	4	−0.86 ± 0.07	8.16 ± 0.12
PGA_1 (2.0 μg/ml)	4	−1.01 ± 0.08	8.03 ± 0.03
PGE_2 (0.2 μg/ml)	4	−0.87 ± 0.09	8.01 ± 0.11

The slope and pA_2 values were estimated graphically from the Arunlakshana-Schild plot. There was no significant difference between pA_2s or slopes and the latter did not differ significantly from −1 (Dunnett's t test, $P > 0.05$).

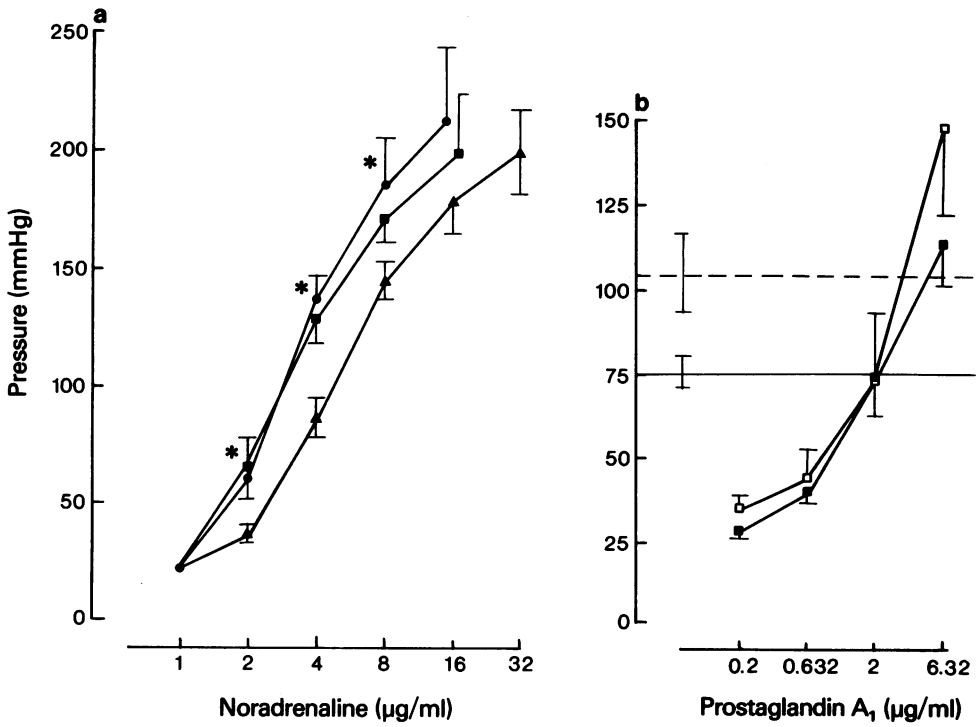

Figure 4 (a) Effect of cocaine (●) 5 μg/ml and (■) 10 μg/ml on vasoconstrictor responses to noradrenaline. The line derived from * points was significantly different from the control (▲) concentration-effect curve (analysis of variance, $P < 0.05$, $n = 4$). Dose-ratio = 2.4 (95% confidence limits from 4.05 to 1.59). (b) The restorative and potentiating effect of prostaglandin A_1 (PGA_1) in the absence (■) and presence (□) of a maximal uptake blocking concentration of cocaine (10 μg/ml, see Figure 4a). The horizontal lines represent the response to approximate EC_{50}s for noradrenaline which were 3.0 μg/ml in the absence of cocaine (solid line) and 2.0 μg/ml with cocaine (broken line). All responses as Figure 2 were obtained in the presence of indomethacin 25 μg/ml. There was no significant difference in position or slope between the two lines (analysis of variance $P > 0.05$, $n = 4$). Vertical bars show the s.e. means.

line may be partly due to blockade of neuronal or extraneuronal uptake was investigated with cocaine and 17-β oestradiol respectively.

Perfusion of tissues with cocaine 5 μg/ml caused a small but significant potentiation of responses to noradrenaline ($P < 0.05$). The dose-ratio was 2.5 with 95% confidence limits from 4.05 to 1.59. The effect of cocaine at 5 μg/ml was maximal since 10 μg/ml caused no further potentiation of noradrenaline responses (Figure 4a). Tyramine at concentrations up to 1 mg/ml failed to cause any vasoconstrictor responses ($n = 3$). When experiments demonstrating the restorative and potentiating effect of PGA_1 were repeated as in Figure 2 in the presence of cocaine (10 μg/ml) there was no significant difference in dose-ratio or slopes compared to the PGA_1 control line ($P > 0.05$) (Figure 4b).

The extraneuronal uptake-blocking drug 17-β oestradiol was perfused through tissues for 10 min before vasoconstrictor responses to noradrenaline were obtained. At a concentration of 10 μg/ml there was a significant decrease in slope of the noradrenaline concentration-effect curve ($P < 0.05$) although the dose-ratio was not significantly different (1.6 with 95% confidence limits from 2.92 to 0.81).

Discussion

This investigation has confirmed that indomethacin depresses the vasoconstrictor responses to noradrenaline (Horrobin *et al.*, 1974) and in addition, shows that the depression is characterized by a concentration-related flattening of the noradrenaline concentration-effect curve. Horrobin *et al.* (1974) also showed that aspirin depressed vasoconstrictor responses and that after complete abolition of the effects of noradrenaline, responsiveness could be restored by PGE_2 but only to about 50% of the control level. It is possible that the concentration of aspirin required to abol-

ish totally responses to noradrenaline also caused effects in addition to inhibition of prostaglandin synthetase, perhaps reducing the contractile capacity of the smooth muscle, and hence reducing the restorative ability of PGE_2. Non-steroid anti-inflammatory drugs (NSAIDs) at higher concentrations also inhibit oxidative phosphorylation and phosphodiesterase (Flower, 1974) and both effects would tend to depress responses to vasoconstrictors. However, the observation in the present study that prostaglandins (excluding PGE_1) restored and, in some cases, potentiated indomethacin-depressed responses is strong evidence in favour of indomethacin having a selective action on prostaglandin sythetase at the concentration used, rather than causing a non-specific depression. This, coupled with the low concentrations of PGE_2 in particular needed to restore indomethacin-depressed responses suggests that endogenous prostaglandins are necessary for the vasoconstrictor action of noradrenaline. One difference in results between this and the study of Horrobin *et al.* (1974) is that PGE_1, although producing only 70% restoration, was effective over the same concentration range as PGE_2 whereas Horrobin *et al.* (1974) found that PGE_1 was 100 times less potent than PGE_2.

A restorative effect was also observed for $PGF_{2\alpha}$ in high concentrations; a finding contrary to that of Horrobin *et al.* (1974). More striking, however, was the observation that $PGF_{2\alpha}$ and additionally PGA_1 and PGA_2 possess a restorative capacity which differs from that of the E prostaglandins. Unlike the E prostaglandins, the PGAs and $PGF_{2\alpha}$ increased the indomethacin-depressed responses to noradrenaline to a level greater than the controls. The emergence of what appears to be two classes of prostaglandin, depending on the type of restorative action, may suggest that there is more than one type of prostaglandin receptor in vascular smooth muscle. The finding also, that in equi-restorative concentrations PGE_2 did not alter normal responses to noradrenaline but PGA_1 potentiated these responses, is further evidence suggesting that there may be different receptor types for prostaglandins in this tissue.

Although the ability of PGA_1 to potentiate normal noradrenaline responses, as well as slightly raising the basal perfusion pressure, was suggestive of an inhibitory action on the neuronal uptake of noradrenaline, this explanation is unlikely. Blockade of neuronal uptake by cocaine caused only a small (2.5-fold) increase in sensitivity to noradrenaline compared with a 4.6-fold potentiation by PGA_1. Moreover, when neuronal uptake was fully blocked by cocaine, responses to noradrenaline could still be depressed by indomethacin and subsequently restored and potentiated by PGA_1. Termination of the effect of noradrenaline in vascular smooth muscle is probably mainly due to enzymatic degradation rather than the result of its inactivation by uptake (Kalsner & Nickerson, 1969). In trial experiments in this and in another study (Leach & Zumani, 1969) the indirectly acting sympathomimetic amine tyramine failed to cause any vasoconstrictor response over a wide range of doses up to 1 mg. It would therefore appear that blockade of neuronal uptake by cocaine is not a very effective means of potentiating noradrenaline in the rat isolated mesenteric blood vessels, a view supported by other authors (Haeusler & Haefely, 1970), and that the restorative and potentiating actions of PGA_1 do not involve blockade of neuronal uptake.

Inactivation of noradrenaline can also involve uptake by effector cells including vascular smooth muscle (Iversen, 1973) and the relative importance of neuronal and extraneuronal uptake mechanisms is believed to vary with the density of adrenergic innervation (Gillespie, 1973). Noradrenaline transported into smooth muscle is largely metabolized by the degradative enzymes, catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO) (Iversen, 1973). PGA_1, PGA_2 and $PGF_{2\alpha}$ may produce their restorative-potentiating effect, in part, by inhibition of the degradative enzymes or blockade of the extraneuronal uptake mechanism. There is no evidence to date to support or refute such a proposal. However, experiments with the extraneuronal uptake blocker 17-β oestradiol showed that this agent depressed rather than potentiated the responses to noradrenaline. No explanation has been offered for this observation.

The mechanism of the depression of noradrenaline responses by indomethacin and the subsequent basic restorative effect of the prostaglandins also remains obscure. Prostaglandins do not appear to alter the affinity of the α-adrenoceptor as was suggested by Horrobin *et al.* (1974). A change in the affinity for the α-adrenoceptor antagonist phentolamine would be expected to accompany any such alteration, but the pA_2, which characterizes different receptors (Schild, 1957), was unaffected by either PGE_2 or PGA_1. This suggests that prostaglandins exert their effects at a site distal to the noradrenaline-α-adrenoceptor interaction. However, it is possible that if the endogenous production of PGE_2 was already producing a maximal effect, that exogenously added PGE_2 would cause no further change in the α-adrenoceptor. This possibility remains unresolved since it was not possible to obtain pA_2 values in the presence of indomethacin because the drug depressed responses to noradrenaline. More certainty can be placed on the lack of effect of PGA_1 on the α-adrenoceptor since the effect of endogenous production cannot have been maximal in this case because exogenous PGA_1 potentiated normal vasoconstrictor responses to noradrenaline (PGE_2 had no effect).

Apart from the non-specific actions of indometha-

cin already mentioned, Northover (1968; 1971) demonstrated that vasoconstrictor responses of the rat isolated perfused anterior mesenteric artery to adrenaline and to calcium ions were prevented or abolished by indomethacin and other NSAIDs. Continued perfusion of the tissue with calcium-containing depolarizing solution (high K^+) caused a persistent rise in perfusion pressure which was rapidly reversed by these agents. The effects were essentially similar to the action of the smooth muscle relaxant, papaverine (Northover, 1968). The EC_{50} of indomethacin for reduction of vasoconstrictor responses to adrenaline and Ca^{2+} was 35 μg/ml, a value similar to that found in this study (about 20 μg/ml) for 50% depression of the responses to noradrenaline. Northover (1968) concluded that indomethacin did not prevent calcium ions from being made available to the contractile proteins, but rather, prevented the calcium ions from causing the contractile proteins to shorten.

A contractile protein has been extracted from vascular smooth muscle which resembles actomyosin from skeletal muscle in that it develops adenosine triphosphatase (ATP-ase) activity and contracts under the influence of calcium ions (Bohr, Filo & Guthe, 1962). It is likely, therefore, that a calcium-dependent ATP-ase exists in vascular smooth muscle which may represent another target for indomethacin inhibition of vasoconstrictor responses. It is interesting to note, that the order of potency of NSAIDs for the abolition of responses to calcium (Northover, 1968) and for the inhibition of prostaglandin synthetase (Flower, 1974) appears to be the same. This evidence suggests at least two possibilities. Either indomethacin may act on a receptor common to prostaglandin synthetase and the calcium-dependent ATP-ase, inhibiting both, or indomethacin inhibits prostaglandin synthetase only, but the ATP-ase requires prostaglandins as essential cofactors additional to calcium ions.

The evidence of Northover (1968) coupled with the restorative action of prostaglandins is suggestive of prostaglandin participation in calcium activation of vascular smooth muscle contraction in rat mesenteric blood vessels. Any interpretation should however, be regarded with caution in view of the multiple effects of indomethacin and other NSAIDs. Depression by indomethacin of the vasoconstrictor action of noradrenaline has also been reported in the rat isolated perfused kidney (Malik & McGiff, 1975; Armstrong *et al.*, 1976) whereas potentiation has been observed in rat skeletal muscle (cremaster) *in vivo* (Messina, Weiner & Kaley, 1975) and rabbit isolated mesenteric blood vessels (Malik *et al.*, 1976). In these vascular beds the effects of prostaglandins at low concentrations are generally opposite to indomethacin. This suggests that prostaglandins are important modulators of vasoconstrictors but that the effect varies between species and regions and may serve the function of the particular vascular bed.

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