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# PROSTAGLANDIN ACTION, RELEASE AND INACTIVATION BY RAT ISOLATED PERFUSED MESENTERIC BLOOD VESSELS

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**1** The following experiments were undertaken to confirm that prostaglandin is necessary for noradrenaline to exert its full vasoconstrictor effect in rat mesenteric blood vessels. Prostaglandin release and inactivation were also studied.

**2** The cyclo-oxygenase inhibitor, 5, 8, 11, 14-eicosatetraynoic acid caused a significant depression of the concentration-effect curve to noradrenaline. As with indomethacin, responses were restored to control levels by prostaglandin $E_2$ ($PGE_2$) but $PGE_2$ did not restore responses to noradrenaline depressed by papaverine.

**3** $PGE_2$-like activity was released from tissues at rest, equivalent to $50 \pm 20$ pg $PGE_2$/min. The substance was probably a stable prostaglandin since activity remained on acidifying and extracting into chloroform. The increase in release stimulated by noradrenaline was reduced below resting values by indomethacin.

**4** There was a net loss of $7 \pm 1$ and $1 \pm 0.2$ ng $PGE_2$/min from tissues perfused with 40 and 4 ng/min $PGE_2$ respectively. No uptake occurred at lower $PGE_2$ perfusion rates.

**5** When indomethacin was used to depress responses to noradrenaline 15(S)-15-methyl $PGE_2$ methyl ester was 12 times more potent than $PGE_2$ in restoring responses to control values. The cyclic endoperoxide analogue U-46619 caused only partial restoration of indomethacin-depressed responses to noradrenaline but increased perfusion pressure at 2 ng/ml and above.

**6** The results confirm that endogenous prostaglandin release, possibly of $PGE_2$, is obligatory to the full vasoconstrictor effect of noradrenaline. Noradrenaline increases the amount of prostaglandin released which may then be taken up and inactivated by 15-hydroxy prostaglandin dehydrogenase or $\beta$-oxidase. U-46619 may mimic both $PGE_2$ and thromboxane $A_2$.

## Introduction

There are many reports indicating that prostglandins exert a modulating influence on the effect of vasoconstrictors. In general, prostaglandins produced by blood vessels counteract vasoconstrictors. However, rat mesenteric blood vessels differ in this respect where it appears that endogenous prostaglandin synthesis is essential for noradrenaline to produce its full vasoconstrictor effect. The evidence for this conclusion is that indomethacin causes a large inhibition of responses to noradrenaline and that certain prostaglandins are able to restore these responses (Horrobin, Manku, Karmali, Nassar & Davis, 1974; Coupar & McLennan, 1978). However, indomethacin may alter responses by its ability to inhibit 15-hydroxy prostaglandin dehydrogenase, phosphodiesterase and oxidative phosphorylating enzymes as well as cyclo-oxygenase (Flower, 1974). 5,8,11,14-Eicosatetraynoic acid (ETA, Figure 1a) is a cyclo-oxygenase inhibitor which is chemically unrelated to indomethacin and has, therefore, been used to confirm that endogenous prostaglandin synthesis is necessary for maintaining the full vasoconstrictor effect of noradrenaline. Prostaglandin release has also been measured to correlate release with vasoconstrictor changes produced by noradrenaline and indomethacin. Further attempts to confirm the importance of prostaglandins in rat mesenteric blood vessels have been made by measuring the uptake of $PGE_2$ by tissues and by determining the possible presence of 15-hydroxy prostaglandin dehydrogenase or $\beta$-oxidase by the use of 15(S)-15-methyl prostaglandin $E_2$ methyl ester which is not a substrate for these enzymes (Weeks, Ducharme, Magee & Miller, 1973; Strand, Miller & McGiff, 1974).

Different prostaglandins vary in their ability to restore indomethacin-depressed responses to noradrenaline (Coupar & McLennan, 1978) but the effect of the cyclic endoperoxides from which the prostaglandins, prostacyclin and thromboxanes are formed have not been investigated. The effect of the stable

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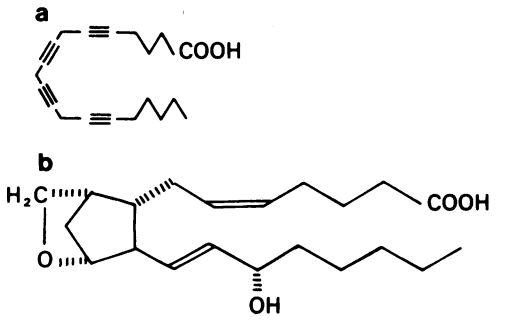


**Figure 1** Structural formulae of (a) 5,8,11,14-eicosatetraynoic acid (ETA) and (b) 15(S)-hydroxy-11.9-(epoxymethano)prosta-5Z,13E-dienoic acid (U-46619).

endoperoxide analogue U-46619 (Figure 1b) has also been studied.

## Methods

Male rats of the Sprague-Dawley strain were used, having a mean weight of 245 g (range 200 to 300 g).

*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). The superior mesenteric artery was perfused 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 off 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, connected to the pump by a tube each converging on a Y-piece, thus enabling perfusion from either reservoir by occluding the opposite tube. All experiments were preceded by an equilibration period of 1 h. Tissues were exposed to only one type of prostaglandin each and responses to noradrenaline were measured as changes in perfusion pressure (1 mmHg = 133 Pa).

*Collection of samples perfused through mesenteric blood vessels*

Cannulated mesenteric blood vessel preparations were immersed in a 30 ml organ bath maintained at 37°C and filled with liquid paraffin to facilitate collection of the perfusate. The perfusion fluid fell to the bottom of the bath and was continuously removed by syphoning. The dead space in the bottom of the organ bath and syphon tube was 1.5 ml.

Prostaglandin-like activity was recovered from the perfusates by a shortened extraction procedure (Unger, Stanford & Bennett, 1971). The samples were acidified to pH 3.5 to 4 with 3% v/v formic acid and extracted with two equal volumes of fraction distilled chloroform. The chloroform was removed at 35°C by use of a rotary evaporator. An additional 10 ml of chloroform was evaporated from the sample to aid removal of formic acid and the residue was blown with $O_2$-free $N_2$ to remove the last traces of formic acid. The dry residue was dissolved in Krebs-Henseleit solution.

*Assay of prostaglandin $E_2$*

Prostaglandin $E_2$ ($PGE_2$) and $PGE_2$-like activity ($PGE_2$ equivalents) was bioassayed against authentic $PGE_2$ on superfused rat fundus strips by a method similar to that described by Ferreira & De Souza Costa (1976). Fundus strips were suspended in an organ bath containing liquid paraffin maintained at 37°C and were superfused down their serosal surface from a fine polyethylene tube (PP 10) acting as a connecting thread. The superfusing fluid was oxygenated Krebs-Henseleit solution with added glucose and containing also a mixture of antagonists (atropine, mepyramine, phenoxybenzamine 0.1 μg/ml, methysergide, propranolol 0.2 μg/ml and indomethacin 2 μg/ml) to increase precision and sensitivity. The superfusion flow rate was maintained at 0.2 ml/min by a peristaltic pump (Cole-Palmer, Masterflex). Samples and standard solutions were drawn up directly for 1 to 1.5 min leaving small air bubbles to separate them from the rest of the superfusing solution. In this way, with sensitive strips, it was possible to detect as little as 1 pg/ml $PGE_2$.

*Statistical analysis*

A 6 point analysis was used for comparing pairs of curves for differences in position and slope with an analysis of variance on the results (Colquhoun, 1971). Paired and Dunnett's $t$ tests were used to compare differences between individual means. In all experiments, statistical significance was taken to be when $P < 0.05$.

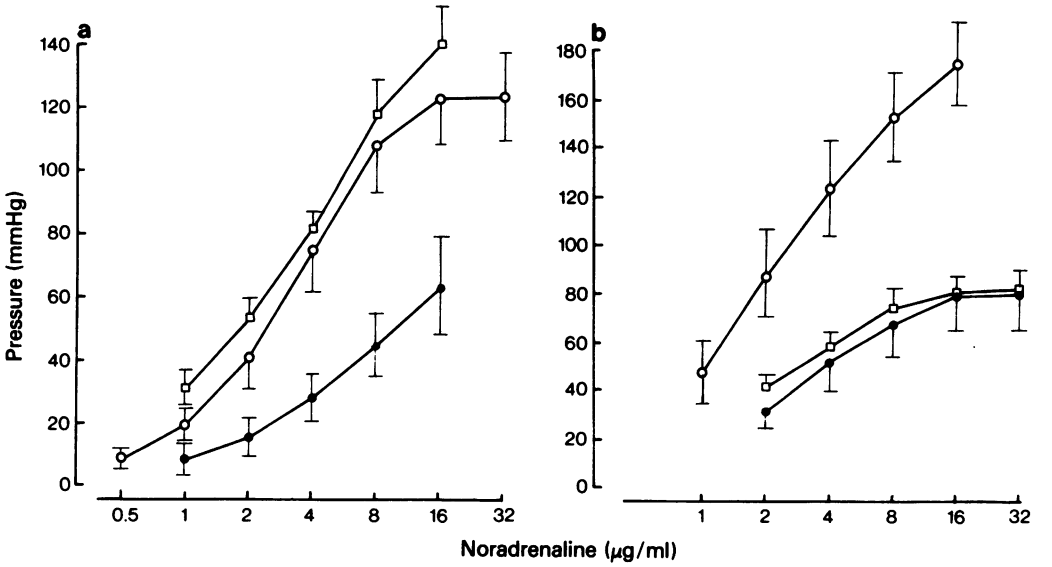


**Figure 2** (a) The ordinate scale represents the maximal increase in pressure produced in response to 10 s infusions of noradrenaline. Responses were produced at 2 min intervals and curves obtained every 20 min. The control curve (○) was shifted to the right and decreased in slope by ETA (6 μg/ml for 20 min) (●). The concentration-effect curve was restored to control values in the continued presence of ETA by prostaglandin $E_2$ ($PGE_2$) (0.2 μg/ml for 20 min) (□). (b) The control curve (○) was shifted to the right by papaverine (1.6 μg/ml for 20 min) (●) but was not restored to control values by $PGE_2$ (0.2 μg/ml) (□). Each point on the curves is the mean from 5 tissues. The bars represent s.e. means.

*Drugs*

Stock solutions of prostaglandins were prepared at concentrations of 10 mg/ml in absolute ethanol. These were stored at −20°C and warmed to room temperature as required. Working solutions of prostaglandins at 1 mg/ml were prepared as required by diluting into 0.2 mol/l phosphate buffer ($NaH_2PO_4.H_2O$ 11 g/l and $Na_2HPO_4.7H_2O$ 8.45 g/l). Fresh solutions of indomethacin and ETA were prepared at a concentration of 10 mg/ml as required. Indomethacin was dissolved in 0.5% w/v $Na_2CO_3$ and then quickly diluted to the required strength with Krebs-Henseleit solution. ETA was dissolved in 1/3 part of absolute ethanol and then 2/3 parts of 0.5% w/v $Na_2CO_3$ were added. The sources of drugs used were: atropine sulphate (Sigma); 5,8,11,14-eicosatetraynoic acid (Roche); indomethacin (Merck, Sharp & Dohme); mepyramine maleate B.P. (May & Baker); methysergide hydrogen maleinate (Sandoz); (−)-noradrenaline bitartrate, (Levophed, Winthrop); papaverine hydrochloride, B.P. (Macfarlan Smith); pentobarbitone sodium, (Sagital, May & Baker); phenoxybenzamine hydrochloride (Smith Kline & French); prostaglandins (PG)$E_2$, 15(S)-15-methyl $PGE_2$ methyl ester and (15)S-hydroxy-11, 9 (epoxymethano) prosta 5Z, 13E-dienoic acid (U-46619) (Upjohn); propranolol hydrochloride (ICI).

## Results

*Effect of ETA on the noradrenaline concentration-effect curve*

ETA at 6 μg/ml did not alter the resting perfusion pressure but caused a significant shift to the right ($P < 0.05$) and significant reduction in slope ($P < 0.05$) of the concentration-effect curve for noradrenaline. Perfusion of tissues with $PGE_2$ (0.2 μg/ml) included in the Krebs-Henseleit-ETA solution produced a recovery of the noradrenaline concentration-effect curve to normal values (Figure 2a). Papaverine at 1.6 μg/ml also caused a significant shift to the right ($P < 0.05$) of the concentration-effect curve to noradrenaline. However, $PGE_2$ (0.2 μg/ml) did not restore responses to noradrenaline in this case and the concentration-effect curve remained significantly shifted to the right ($P < 0.05$) and significantly reduced in slope ($P < 0.05$) compared to the control curve (Figure 2b).

*Prostaglandin $E_2$-like release*

The extraction method achieved complete recovery of $PGE_2$ (109% extraction of 10 ng $PGE_2$ in 10 ml; range 100 to 130%, $n = 5$) as reported by Unger *et al.* (1971) and no partitioning of $PGE_2$ occurred into the liquid paraffin.

The samples collected from tissues perfused with Krebs-Henseleit alone at 1 h (resting release) and those perfused with added noradrenaline contained $PGE_2$-like activity after acidification and extraction. The resting release from tissues was $50 \pm 20$ pg $PGE_2$ equivalents/min ($n = 7$). An approximate $EC_{50}$ of noradrenaline (2 μg/ml) was then added to the perfusate and a sample collected 2 min later for 5 min. The baseline perfusion pressure increased from $22 \pm 3$ to $116 \pm 10$ mmHg ($n = 7$) and there was a significant concurrent increase in $PGE_2$-like release to $430 \pm 160$ pg $PGE_2$ equivalents/min ($n = 7$, paired $t$ test, $P < 0.05$). At the end of the collection, the perfusing solution was changed to Krebs-Henseleit containing indomethacin (25 μg/ml) for 10 min and then to indomethacin (25 μg/ml) plus added noradrenaline (2 μg/ml) and a sample collected for 5 min, 2 min after starting this final perfusion. Indomethacin reduced the constrictor response of noradrenaline to $30 \pm 4$ mmHg ($n = 6$) and it also blocked the $PGE_2$-like release stimulated by noradrenaline. $PGE_2$-like efflux was undetectable from 3 such tissues (<2 pg/min) and averaged only $12 \pm 4$ pg $PGE_2$ equivalents/min from 3 others.

*Prostaglandin uptake/inactivation*

The effluent from tissues perfused with increasing concentrations of $PGE_2$ was collected in order to detect any removal of $PGE_2$ by the tissues indicating the presence of an uptake or inactivation process. Perfusates were collected for 5 min after 10 min equilibration to increasing concentrations of $PGE_2$. Perfusion fluid without $PGE_2$ was also collected at the start of experiments to act as a control. The samples were diluted if necessary and assayed directly without extraction and reconstitution in Krebs-Henseleit solution. Analysis of variance showed that the infusion rates of $PGE_2$ had a statistically significant effect on the mean net amount of $PGE_2$-like release or $PGE_2$ uptake by tissues ($P < 0.001$). However, loss of $PGE_2$ from perfusates became statistically significant only when $PGE_2$ was perfused at 40 ng/min compared to control (Dunnett's $t$ test, $P < 0.001$). There was virtually no net loss of $PGE_2$ when tissues were perfused with 0.4 ng $PGE_2$/min. $PGE_2$-like activity was released from tissues perfused with $PGE_2$ at the lowest rate of 40 pg $PGE_2$/min. The amount of this release in addition to the infusion rate of 4 pg $PGE_2$/min was similar to the resting value of $PGE_2$-like release (Dunnett's $t$ test, $P > 0.05$) (Table 1).

*The effect of prostaglandins in the presence of indomethacin*

Two similar responses (within 5 mmHg) were obtained to an $EC_{50}$ of noradrenaline (2 μg/ml), first in the absence, then after 15 min equilibration with indomethacin (25 μg/ml). This concentration caused $72 \pm 3\%$ ($n = 21$) depression of responses to noradrenaline. While maintaining indomethacin in the perfusate, prostaglandins were perfused through the tissues in progressively increasing concentrations, and responses to noradrenaline were obtained at each level. Each prostaglandin concentration was maintained in the perfusate for 10 min before responses to noradrenaline were obtained. All three prostaglandins studied had some degree of restorative activity on indomethacin-depressed responses to noradrenaline. Neither $PGE_2$ nor 15(S)-15-methyl $PGE_2$ methyl ester changed baseline perfusion pressure in the concentration ranges used (up to 200 and 20 ng/ml respect-

**Table 1** Uptake/inactivation of prostaglandin $E_2$ ($PGE_2$) and release of $PGE_2$-like activity by tissues perfused with $PGE_2$ at different rates

| Amount of $PGE_2$ perfused (ng/min) | — | 0.04 | 0.4 | 4 | 40 |
|---|---|---|---|---|---|
| Amount of $PGE_2$ (ng) lost from (−) or released into (+) perfusate/min | $+0.03 \pm 0.02$* | $+0.08 \pm 0.04$ | $+0.01 \pm 0.05$ | $-1.1 \pm 0.02$ | $-7.2 \pm 1.2$* |
| % of original $PGE_2$ lost from (−) or released into (+) perfusate/min | — | $+200 \pm 99$ | $2.5 \pm 13$ | $-27.5 \pm 5$ | $-18 \pm 3$ |
| Number of experiments | 5 | 6 | 9 | 5 | 5 |

Perfusates were collected before and then during perfusion of tissues with each of 4 increasing concentrations of $PGE_2$. There was a net loss of $PGE_2$ (−) from the perfusion fluid at the 2 higher perfusion rates of $PGE_2$. However, $PGE_2$-like activity was released into the perfusate (+) in addition to the amount of $PGE_2$ perfused at the lower rates.

*$P < 0.001$, Dunnett's $t$ test.

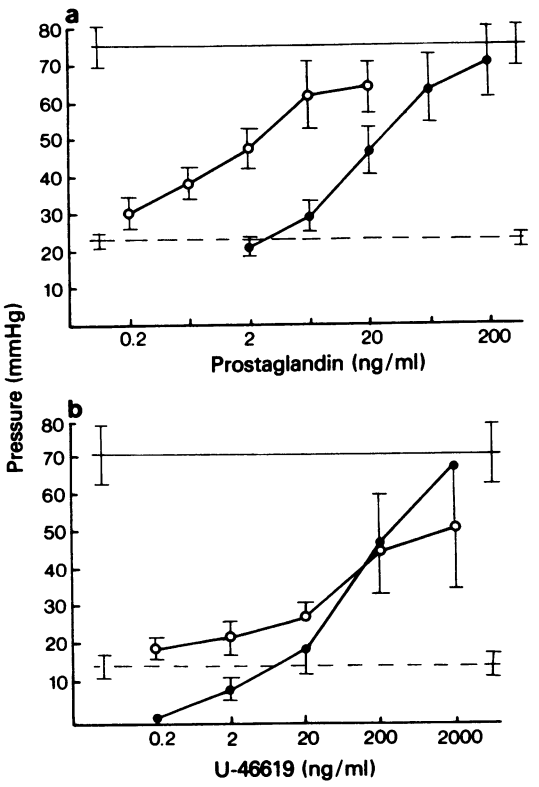


**Figure 3** Effect of (a) prostaglandin $E_2$ and 15(S)-15-methyl prostaglandin $E_2$ methyl ester and (b) U-46619 on indomethacin-depressed responses to noradrenaline. The upper horizontal lines represent the mean control responses to an approximately $EC_{50}$ of noradrenaline (2 μg/ml, $n = 21$) and the lower hatched lines represent the mean response to noradrenaline after 15 min equilibration of tissues with indomethacin (25 μg/ml, $n = 21$). (a) 15(S)-15-methyl prostaglandin $E_2$ ($n = 6$) (○) was 12 times more potent than prostaglandin $E_2$ ($n = 6$) (●) in restoring indomethacin-depressed responses to noradrenaline to normal (95% confidence limits 7-25). The concentration-effect lines did not differ significantly in slope ($P > 0.05$). (b) U-46619 caused partial restoration of indomethacin-depressed responses to noradrenaline ($n = 7$) (○) but also caused a direct rise in perfusion pressure ($n = 6$) (●).

ively) but both restored noradrenaline-induced responses to control values. 15(S)-15-methyl $PGE_2$ methyl ester, which is not a substrate for 15 hydroxyprostaglandin dehydrogenase and β-oxidase, was 12 times more potent than $PGE_2$ in this respect (potency ratio 12, 95% confidence limits 7–25) (Figure 3a).

Low concentrations of U-46619, which is a stable epoxymethano derivative of $PGH_2$ produced partial restoration of indomethacin-depressed responses to noradrenaline. However, at concentrations above 20 ng/ml U-46619 itself produced dose-related rises in perfusion pressure (Figure 3b).

## Discussion

The suppression of responses to noradrenaline by 5,8,11,14-eicosatetraynoic acid (ETA), as with indomethacin provides additional evidence that prostaglandin deficiency in rat mesenteric blood vessels causes loss of responsiveness to noradrenaline. ETA was used since it is more selective than indomethacin which may inhibit 15-hydroxy-prostaglandin dehydrogenase, phosphodiesterase and enzymes involved in oxidative phosphorylation (Flower 1974). These potential effects of indomethacin may alter responses to noradrenaline additionally to cyclo-oxygenase inhibition. ETA on the other hand is a false substrate for cyclo-oxygenase causing irreversible inhibition of the enzyme (Flower, 1974). It has also been shown to abolish prostaglandin synthesis in rat pancreas (Hamamdzic & Malik, 1977).

Further confirmation that prostaglandin is required by rat mesenteric blood vessels to maintain responsiveness to noradrenaline has been obtained by extraction and bioassay of material released into the perfusion fluid of tissues. These experiments detected a small resting release of $PGE_2$-like activity from the blood vessels which was increased approximately 10 fold by an $EC_{50}$ concentration of noradrenaline. The low resting $PGE_2$-like release may result from leakage of a larger amount of prostaglandin produced continuously in the vascular wall permitting noradrenaline to initiate constriction. When noradrenaline causes constriction more prostaglandin is produced, possibly to intensify the vasoconstrictor response. The finding that indomethacin caused a large reduction in both noradrenaline-stimulated $PGE_2$-like release below resting values and in the vasoconstrictor response to noradrenaline strongly suggests that noradrenaline is dependent on prostaglandin release in order to produce vasoconstriction.

The prostaglandin responsible for maintaining responsiveness may be $PGE_2$ since it is the most potent naturally occurring prostaglandin yet studied that is able to restore completely indomethacin-depressed responses to noradrenaline (Horrobin *et al.*, 1974; Coupar & McLennan, 1978). The present results indicate also that $PGE_2$ exerts a selective restorative action since it only restores responses to noradrenaline depressed by the cyclo-oxygenase inhibitors indomethacin and ETA but not responses depressed by the non-selective smooth muscle relaxant papaverine.

The essential role of prostaglandin in these blood vessels may necessitate an inactivation or uptake sys-

tem for terminating action. One way of determining this possibility was by the use of 15(S)-15-methyl $PGE_2$ methyl ester which is not a substrate for 15-hydroxy prostaglandin dehydrogenase (Weeks *et al.*, 1973) and should be resistant to $\beta$-oxidation of the carboxylic acid side chain. 15(S)-15-methyl $PGE_2$ methyl ester was found to be 12 times more potent than $PGE_2$ in restoring indomethacin-depressed responses to noradrenaline which may indicate the presence of significant amounts of 15-hydroxyprostaglandin dehydrogenase or $\beta$-oxidase within the blood vessels. However, it is also possible that the two structural changes in the $PGE_2$ molecule may alter pharmacological activity. For example, $PGE_2$ methyl ester is less potent than $PGE_2$ in contracting the intestine while 15-methylation of the methyl ester restores potency again (Weeks *et al.*, 1973; Strand *et al.*, 1974). It has not yet been determined whether the increased potency of 15-methylprostaglandin $E_2$ is caused solely by its resistance to 15-hydroxyprostaglandin dehydrogenase or due also to increased affinity at its receptor.

Another approach to the problem of determining whether the action of $PGE_2$ might be terminated within the blood vessels was by perfusion of tissues with known amounts of $PGE_2$ and assaying the amount remaining in the perfusate. The present experiments showed, surprisingly, that inactivation or uptake of $PGE_2$ occurred only above a certain rate of perfusion. No loss of $PGE_2$ occurred on perfusing tissues at the rate of 0.4 ng $PGE_2$/min. Conversely there was even an apparent net release of $PGE_2$-like activity at the lowest infusion rate (40 pg $PGE_2$/min). This could indicate that the exogenous $PGE_2$ passed through the tissues without influencing the endogenous resting release of $PGE_2$-like activity. It should be noted that the concentration at which inactivation/uptake of perfused $PGE_2$ is initiated is not high and correlates well with the effect of exogenous $PGE_2$ on the vascular smooth muscle. When 4 ng $PGE_2$/min is perfused its absolute removal rate is slow and the amount is not sufficient to restore indomethacin-depressed responses to noradrenaline. But at 40 ng $PGE_2$/min (20 ng/ml) there is significant inactivation/uptake and approximately 50% restoration of indomethacin-depressed responses. These results, therefore indicate that significant inactivation/uptake of $PGE_2$ from the blood vessel lumen occurs only when the concentration in the lumen is sufficient to produce the physiological effect. At this concentration either uptake or enzymatic inactivation or both may be activated possibly as a protective mechanism. Excessive amounts of E-type prostaglandins reaching the mucosa stimulate fluid and electrolyte loss from the rat intestine (Coupar & McColl, 1975).

Prostaglandins differ in their restorative effects on indomethacin-depressed responses to noradrenaline. One group ($PGE_1$ and $E_2$) restore responses to control values while others ($PGA_1$, $A_2$ and $F_{2\alpha}$), acting at higher concentrations, increase responses above control levels (Coupar & McLennan, 1978). In this respect it was of interest to investigate the effect of the cyclic endoperoxide $PGH_2$, since it occupies a pivotal position in prostaglandin and thromboxane synthesis and may possess intrinsic activity of its own. U-46619 is an epoxymethano derivative of $PGH_2$ and was used because it is chemically stable and is not biotransformed to $PGE_2$ (Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975). U-46619 was pharmacologically active possessing weak $PGE_2$-like activity in restoring indomethacin-depressed responses to noradrenaline. However, any potential for fully restoring responses was masked by its direct vasoconstrictor action. Since U-46619 aggregates platelets and increases airways resistance (Beckmann & Leovey, 1976) as well as constricting blood vessels (Alter, Kot, Ramwell, Rose & Shnider, 1977) it may mimic the effects of thromboxane $A_2$ more closely than $PGH_2$.

To date, studies of the effects of non-steroid anti-inflammatory drugs and prostaglandins in rats indicate that the rat is unusual compared to other experimental animals such as dogs, cats and rabbits in having a predominance of blood vessels where endogenous prostaglandin synthesis (possibly $PGE_2$) facilitates noradrenaline-induced vasoconstriction. These blood vessels are those of mesentery (Horrobin *et al.*, 1974; Malik & McGiff, 1974; Malik, Ryan & McGiff, 1976; Manku & Horrobin, 1976; Coupar & McLennan, 1978) hindlimb (Kondo, Misumi, Okuno, Nakamura, Saruta & Kato, 1978) and kidney (Armstrong, Blackwell, Flower, McGiff & Mullane, 1975; Malik & McGiff, 1975). High concentrations of $PGE_2$ even cause vasoconstriction in the rat kidney (Armstrong *et al.*, 1975; Malik & McGiff, 1975). It is therefore, becoming evident that human tissue should be used to determine which would be the most appropriate experimental animals for investigating the potential clinical effects of new drugs acting on the prostaglandin system of blood vessels.

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