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EVIDENCE AGAINST THE INVOLVEMENT OF PROSTAGLANDINS IN THE VASOCONSTRICTOR ACTION OF CALCIUM ION IN RAT MESENTERIC BLOOD VESSELS

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1 Prostaglandin E_2 (PGE_2) has been claimed to be essential to the vasoconstrictor action of noradrenaline in rat mesenteric blood vessels. Since noradrenaline acts by releasing intracellular calcium, experiments have been performed using the perfused rat superior mesenteric artery preparation to determine whether prostaglandin synthesis is necessary for the direct vasoconstrictor action of calcium.

2 The cyclo-oxygenase inhibitors, indomethacin and 5,8,11,14-eicosatetraynoic acid (ETA), inhibited responses to noradrenaline and calcium but both were less effective in inhibiting the response to calcium than to noradrenaline.

3 PGE_2 (6 ng–20 μg/ml) failed to overcome the inhibitory effect of indomethacin (62 μg/ml) and ETA (10 μg/ml) on the response to the EC_{50} of Ca^{2+} (100 μg/ml). The EC_{50} of Ca^{2+} did not significantly increase PGE_2-like release by the blood vessels from the resting value of 19 ± 8 pg of PGE_2 equivalents/min.

4 PGA_1 (6 μg/ml) and the thromboxane A_2 agonist, U-46619 (200 ng/ml), both caused full restoration of indomethacin-depressed responses to calcium, but did not restore responses depressed by ETA. U-46619 (200 ng/ml) also reversed the inhibitory effect of papaverine (4 μg/ml) and caused a 1.6 fold potentiation of Ca^{2+} responses.

5 The results do not support the hypothesis that prostaglandin synthesis is essential to the vasoconstrictor action of Ca^{2+} in rat mesenteric blood vessels.

Introduction

There is some evidence which suggests that prostaglandin E_2 (PGE_2) is essential to the vasoconstrictor action of noradrenaline in rat mesenteric blood vessels. For example, noradrenaline increases the release of PGE_2-like activity from these blood vessels (Wolfe, Rostworowski & Manku, 1979; Coupar, 1980a), and indomethacin, within and above the concentration range known to inhibit cyclo-oxygenase selectively (Flower, 1974), causes a large depression of the vasoconstrictor response to noradrenaline (Horrobin, Manku, Karmali, Nassar & Davies, 1974; Coupar & McLennan, 1978). This indomethacin-induced depression of the response to noradrenaline is associated with a concurrent abolition of PGE_2-like release, and is overcome by adding exogenous PGE_2 to the perfusion fluid (Coupar, 1980a).

The mechanism by which PGE_2 maintains or restores the response to noradrenaline in rat mesenteric blood vessels is not known but there are several potential sites of action in the sequence of excitation-contraction coupling. For example, noradrenaline produces vasoconstriction mainly by releasing intracellular Ca^{2+} from a binding site (Waugh, 1962; Cuthbert & Sutter, 1965; Jhamandas & Nash, 1967; Hudgins & Weiss, 1968) to remove the inhibitory influence of the troponin C-like regulatory protein on the contractile proteins (Perry & Grand, 1979). The regulatory protein may be calmodulin which has been isolated from vascular and other smooth muscles (Perry & Grand, 1979; Grand, Perry & Weeks, 1979).

In view of the fact that Ca^{2+} is the fundamental cation responsible for coupling excitation to contraction, experiments have been undertaken to investigate whether prostaglandins sustain the effect of noradrenaline by acting as a necessary co-factor or intermediate to the vasoconstrictor action of Ca^{2+} itself. Part of this work has been presented to the Australian Physiological and Pharmacological Society (Coupar, 1980b).

Methods

Mesenteric blood vessels of male Sprague-Dawley rats (weight range of 200–300 g) were prepared for perfusion as described previously (McGregor, 1965;

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Coupar & McLennan, 1978). The superior mesenteric artery was perfused at a flow rate of 2 ml/min (Masterflex peristaltic pump) with pregassed (5% CO_2 in O_2) solution maintained at 37°C, unless otherwise stated. For experiments involving noradrenaline-induced constriction a Krebs-Henseleit solution with added dextrose was used of the following composition (g/l): NaCl 6.92, KCl 0.32, $MgSO_4.7H_2O$ 0.29, $KH_2PO_4.2H_2O$ 0.16, $NaHCO_3$ 2.1, $CaCl_2$ 0.28 and dextrose 2. A Ca^{2+}-free/K^+-rich depolarizing solution (Northover, 1968) of the following composition (g/l) was used for experiments involving Ca^{2+}-induced constriction: K_2SO_4 16, $KHCO_3$ 1, $MgCl_2.6H_2O$ 0.215, $NaH_2PO_4.2H_2O$ 0.65, dextrose 1.02. In experiments on the effect of Ca^{2+}, the tissues were pretreated with phenoxybenzamine (1 μg/ml for 10 min) in order to block the effect of noradrenaline released by Ca^{2+}. Responses to Ca^{2+} were then obtained by adding $CaCl_2$ to the depolarizing solution and these were larger and more consistent than those induced by adding $CaCl_2$ to Krebs-Henseleit solution. This was expected, since depolarization of the vascular smooth muscle membrane increases its permeability to Ca^{2+} (Briggs, 1962). Also, the solution permitted relatively large amounts of $CaCl_2$ to be added without Ca^{2+} salt precipitation as occurs in Krebs-Henseleit solution.

Constrictor responses were measured as increases in perfusion pressure and were recorded with a Statham P23 AC pressure transducer connected to a Grass polygraph (model 79c). In all experiments an equilibration period of 1 h was allowed before exposure of tissues to the drugs used. Tissues were exposed to only one prostaglandin and/or cyclo-oxygenase inhibitor each.

Release of prostaglandin E_2-like activity from perfused mesenteric blood vessels

Tissues were suspended in a 30 ml organ bath maintained at 37°C. The perfusate was collected under liquid paraffin and was continuously removed by a siphon tube. Prostaglandin-like activity was recovered from the perfusate by extraction (Unger, Stamford & Bennett, 1971) and was bioassayed against authentic PGE_2 on superfused rat fundus strips, equilibrated with a mixture of antagonists (atropine, mepyramine, phenoxybenzamine 0.1 μg/ml, methysergide, propranolol 0.2 μg/ml, indomethacin 2 μg/ml) by previously described methods (Coupar, 1980a).

Statistical analysis

A 6 point bioassay analysis was used for comparing the linear regions in pairs of log concentration-effect lines for differences in position and slope (Colquhoun, 1971). A linear regression analysis was used for calculation of EC_{50} and IC_{50} (Moore, Shirley & Edwards, 1972). Results were considered statistically significant when P was less than 0.05.

Drugs

Stock solutions of prostaglandins were prepared at concentrations of 10 mg/ml in absolute ethanol. Working solutions of prostaglandins (1 mg/ml) were prepared as required by diluting the stock solution with 0.2 mol/l phosphate buffer. Solutions of indomethacin and 5,8,11,14-eicosatetraynoic acid (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. ETA was dissolved in 1 volume of absolute ethanol, and then 2 volumes of 0.5% w/v Na_2CO_3 were added. The sources of drugs were: atropine sulphate (Sigma); 5,8,11,14-eicosatetraynoic acid (ETA; Roche); indomethacin (Merck, Sharp & Dohme); mepyramine maleate B.P. (May & Baker); methysergide hydrogen maleinate (Sandoz); (−)-noradrenaline ·bitartrate (Levophed, Winthrop); papaverine hydrochloride (Macfarlan Smith); phenoxybenzamine hydrochloride (Smith, Kline & French); prostaglandins (PG)E_2, A_1 and (15)S-hydroxy-11,9(epoxymethano)prosta-5Z, 13E-dienoic acid (U-46619; Upjohn); propranolol hydrochloride (ICI). Pilot experiments showed that ethanol in the concentrations used for preparing solutions of PGE_2 and A_1, U-46619 and ETA did not affect constrictor responses to noradrenaline and Ca^{2+}.

Results

Effect of indomethacin and 5,8,11,14-eicosatetraynoic acid on noradrenaline- and calcium-induced responses

The basal perfusion pressure of tissues perfused with Krebs-Henseleit solution was 17 ± 1 mmHg (n = 11) and 36 ± 1 mmHg (n = 31) in tissues perfused with the Ca^{2+}-free/K^+-rich depolarizing solution. Perfusion of noradrenaline produced a fast rise in pressure. The constrictor effect was maximal within 10 s and the approximate EC_{50} of noradrenaline was 2 μg/ml. Vasoconstrictor responses to Ca^{2+} were slower than to noradrenaline, with a maximal effect reached within 1.5 min and an approximate EC_{50} of 100 μg/ml.

To test the inhibitory effect of indomethacin, constrictor responses were obtained to the EC_{50}s of noradrenaline and Ca^{2+}. Increasing concentrations

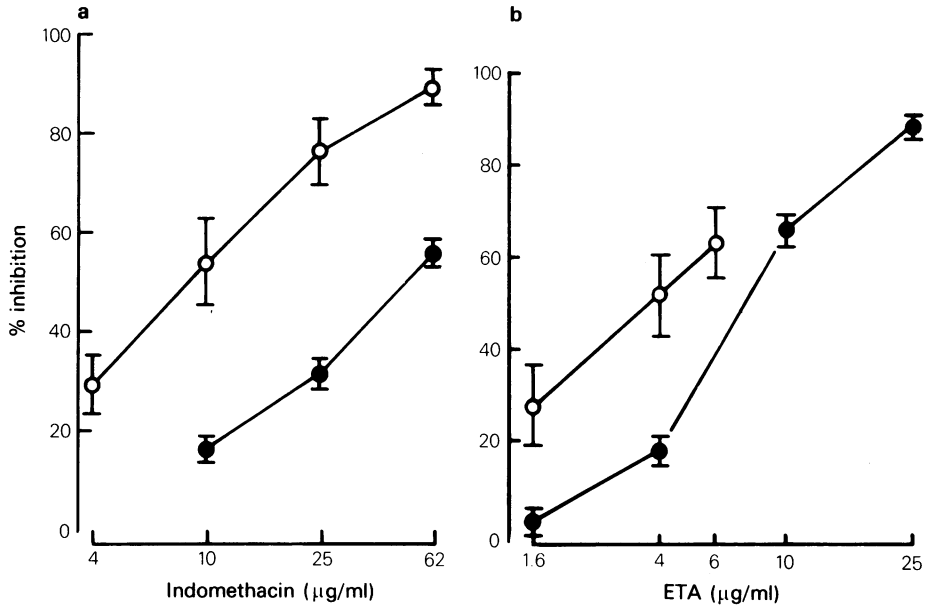

Figure 1 Percent inhibition of EC_{50} constrictor responses to noradrenaline (○) and Ca^{2+} (●) by increasing concentrations of (a) indomethacin and (b) ETA. Each concentration of the cyclo-oxygenase inhibitors was perfused through tissues for 10 min before obtaining EC_{50} responses to the agonists. Noradrenaline was perfused for 10 s and the EC_{50} was 2 μg/ml. Ca^{2+} (as $CaCl_2$) was perfused for 1.5 min, the EC_{50} being 100 μg/ml. Each point is the mean from 5 tissues. The bars represent the s.e.means.

of indomethacin, which did not affect resting perfusion pressure, progressively inhibited the responses to both agonists (Figure 1a). However, 5.5 times more (95% confidence interval (CI) 10–3) indomethacin was required to inhibit Ca^{2+} to the same extent as noradrenaline. The IC_{50} of indomethacin against noradrenaline was 9 μg/ml (95% CI, 37–2) but against Ca^{2+} was 50 μg/ml (95% CI, 160–18). The response of the tissues to Ca^{2+} and to noradrenaline were partially restored after perfusing with indomethacin-free solution. The response to the EC_{50} of Ca^{2+} was reduced to 33 ± 6% of control by 62 μg/ml of indomethacin but this recovered to 86 ± 6% of control after 30 min and 91 ± 6% of control after 1 h ($n = 4$). The same concentration of indomethacin reduced the response to the EC_{50} of noradrenaline to 6 ± 1% of control. The response recovered to 40 ± 3% at 30 min and 56 ± 7% of control at 1 h ($n = 4$).

The results with indomethacin do not agree with those obtained by Manku & Horrobin (1976) who found indomethacin equally effective in inhibiting noradrenaline and Ca^{2+} at 30°C. The experiments were therefore repeated at 30°C to determine whether temperature is the factor responsible for reducing the inhibitory effect of indomethacin against Ca^{2+}. The result, however, was similar to that obtained at 37°C and showed that at 30°C 3.6 times more (95% CI, 4.6–3) indomethacin was needed to inhibit Ca^{2+} to the same extent as noradrenaline.

In further experiments the cyclo-oxygenase inhibitor 5,8,11,14-eicosatetraynoic acid (ETA) was used in place of indomethacin. ETA did not alter resting perfusion pressure but caused inhibition of responses to both noradrenaline and Ca^{2+}, and again Ca^{2+}-induced responses were more resistant to inhibition than those induced by noradrenaline (Figure 1b). The IC_{50} of ETA against noradrenaline (2 μg/ml) was 3.6 μg/ml (95% CI, 39.5–0.4) but against Ca^{2+} (100 μg/ml) was 7.7 μg/ml (95% CI, 14–4).

Release of prostaglandin E_2-like material

The extraction method achieved complete recovery of 1 ng/ml of authentic PGE_2 (96 ± 2.4%, $n = 4$) and no partitioning of PGE_2 occurred into the liquid paraffin. The resting release of PGE_2 from tissues perfused with high K^+/Ca^{2+}-deficient depolarizing solution was 19 ± 8 pg of PGE_2 equivalents/min. An approximate EC_{50} of Ca^{2+} (100 μg/ml) was then perfused through tissues and this increased the perfusion pressure from 27 ± 6 to 167 ± 21 mmHg. However, the amount of PGE_2 activity released remained unaltered at 20 ± 10 pg PGE_2 equivalents/min during the period of sustained constriction ($n = 4$). Using the

same experimental conditions, it was shown previously that rat mesenteric blood vessels release 50 ± 20 pg PGE_2 equivalents/min when perfused with Krebs-Henseleit solution. An approximate EC_{50} of noradrenaline (2 μg/ml) which raised the perfusion pressure from 22 ± 3 to 116 ± 10 mmHg, caused a significant concurrent increase in prostaglandin release to 430 ± 160 pg PGE_2 equivalents/min (Coupar, 1980a).

Effect of prostaglandins on Ca^{2+}-induced responses in the presence of indomethacin, ETA and papaverine

The response to the EC_{50} of Ca^{2+} (100 μg/ml) was depressed by $51 \pm 4\%$ ($n = 13$) after 15 min of perfusion with 62 μg/ml of indomethacin. While maintaining indomethacin in the perfusate, prostaglandins were perfused through tissues at increasing concentrations and responses to the EC_{50} of Ca^{2+} were recorded at each prostaglandin level. Each prostaglandin concentration was maintained in the perfusate for 10 min before inducing constriction with Ca^{2+}. PGA_1, and the chemically stable analogue of PGH_2, U-46619, restored Ca^{2+}-induced responses to pre-indomethacin control values. U-46619 was more potent than PGA_1 but the slope of the log concentration-effect curve was significantly smaller than that of PGA_1 ($P < 0.025$). The results are shown in Figure 2.

U-46619 and PGA_1 caused an additional rise in pressure at the concentrations which fully restored the indomethacin-depressed response to Ca^{2+}. These concentrations were 200 ng/ml of U-46619 which increased basal perfusion pressure by 15 ± 1 mmHg ($n = 5$) and 6 μg/ml of PGA_1 which increased the pressure by 15 ± 2 mmHg ($n = 5$). The lower concentrations of U-46619 and PGA_1, which caused partial reversal of indomethacin-induced response to Ca^{2+}, did not affect resting perfusion pressure.

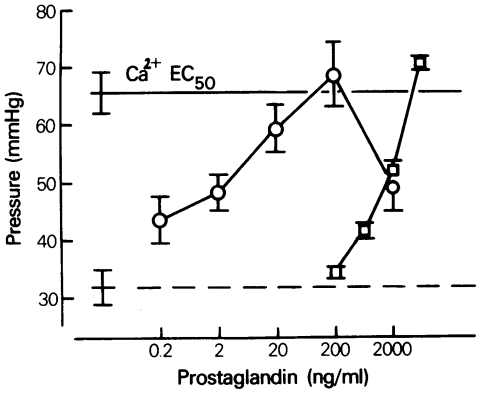

Figure 2 Dose-response curves of U-46619 (○) and prostaglandin A_1 (PGA_1, □) in reversing the response to the EC_{50} of Ca^{2+} depressed by indomethacin. The upper horizontal line represents the mean response to the EC_{50} of Ca^{2+} (100 μg/ml) in the absence of indomethacin; while the lower broken line is the response to Ca^{2+} after 15 min perfusion of tissues with indomethacin (62 μg/ml). Each concentration of U-46619 and PGA_1 was perfused for 10 min before inducing constriction with Ca^{2+} and each point is a mean from 5 tissues. Bars represent s.e.means.

PGE_2 in concentrations ranging from 60 ng to 20 μg/ml, did not cause any rise in perfusion pressure or restoration of the indomethacin-depressed response to Ca^{2+} ($n = 5$).

PGE_2 (6–600 ng/ml) and U-46619 (200 ng/ml) also both failed to restore the response to the EC_{50} of Ca^{2+}-depressed by ETA (10 μg/ml, $n = 4$). PGA_1 was not investigated for possible reversal of ETA-induced inhibition of Ca^{2+}.

Experiments were continued with U-46619 because of the paradoxical results that it was more potent than PGA_1 in reversing indomethacin, but failed to produce any reversal of the inhibition caused by the cyclo-oxygenase inhibitor, ETA. Therefore, U-46619 was next tested to determine whether it reversed the depression of Ca^{2+}-induced responses caused by the non-cyclo-oxygenase inhibitor, papaverine. The results are shown in Figure 3a. The control dose-response curve of Ca^{2+} was significantly reduced in slope ($P < 0.005$) and moved to the right ($P < 0.001$) by perfusing 4 μg/ml of papaverine through the tissues for 15 min. Inclusion of 200 ng/ml of U-46619 in the solution containing papaverine completely restored responses to control levels. Using the same experimental conditions it was shown previously that 200 ng/ml of PGE_2 completely restores indomethacin- and ETA-depressed responses to noradrenaline, but has no effect on responses to noradrenaline depressed by papaverine (Coupar, 1980a).

Effect of U-46619 on the calcium log concentration-effect curve

Since U-46619 was found to have potent actions in restoring indomethacin- and papaverine-depressed responses to Ca^{2+}, further experiments were performed to determine whether this effect was due to potentiation of Ca^{2+}-induced responses, regardless of indomethacin or papaverine. After establishing responses to cumulatively increasing concentrations of Ca^{2+}, the most effective restoring concentration of U-46619 (200 ng/ml) was equilibrated with tissues for 10 min and responses to Ca^{2+} obtained again. U-46619 caused a small but statistically significant 1.6 fold (95% CI, 2.0–1.2) potentiation of Ca^{2+}-induced responses ($P < 0.05$, $n = 6$). The results are shown in Figure 3b.

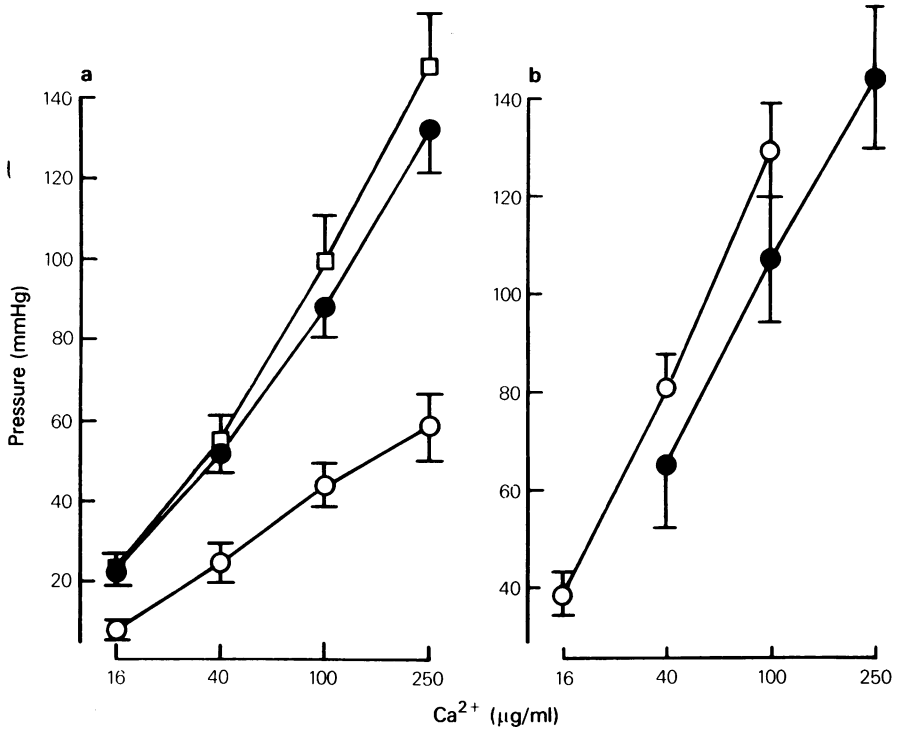

Figure 3 Effect of U-46619 on cumulative dose-response curves to Ca^{2+}. Each concentration of Ca^{2+} was perfused through tissues for 1.5 min and dose-response curves were produced every 20 min. (a) The control curve to Ca^{2+} (●) was significantly reduced in slope and moved to the right after 15 min perfusion of tissues with 4 μg/ml of papaverine (○). Constrictor responses to Ca^{2+} in the continued presence of papaverine were completely restored to normal values by a 10 min perfusion with 200 ng/ml of U-46619 (□) ($n = 5$). (b) The control curve to Ca^{2+} (●) was significantly moved to the left after 10 min perfusion of tissues with 200 ng/ml of U-46619 (○), ($P < 0.05$, $n = 6$). Bars represent s.e.means.

Discussion

The present results show that indomethacin decreases the constrictor response of rat mesenteric blood vessels to both Ca^{2+} and noradrenaline. However, considerably more indomethacin (5.5 times) was required to reduce the response to Ca^{2+} to the same extent as noradrenaline. This is not in agreement with the result of Manku & Horrobin (1976) who found the IC_{50} of indomethacin was approximately 7 μg/ml against both noradrenaline and Ca^{2+}. The similar IC_{50} of indomethacin against the two agonists was part of the evidence which led them to suggest that prostaglandin may be necessary to the action of noradrenaline as well as Ca^{2+}. The different temperatures used in the present study (37°C) and that of Manku & Horrobin (30°C) do not explain the different IC_{50} values for indomethacin against Ca^{2+}. Manku & Horrobin (1976) used Krebs-Henseleit solution containing the normal amount of Ca^{2+}; therefore it is possible that perfusion with high potassium/Ca^{2+}-deficient solution decreases the inhibitory effect of indomethacin against Ca^{2+} by an as yet undetermined mechanism. Alternatively, it is possible that indomethacin may be more effective against bolus doses of Ca^{2+}, as used by Manku & Horrobin (1976), than against equilibrium responses to Ca^{2+} as obtained in this study. For these reasons, direct comparison between this study and the study of Manku & Horrobin should be treated with reservation.

It must be stressed that high concentrations of indomethacin, similar to those used in the present experiments, have been shown to inhibit the accumulation and binding of Ca^{2+} by vascular and other smooth muscles (Northover, 1971; 1972; 1973). Also, similar high concentrations of indomethacin have been shown to inhibit Ca^{2+}-activated ATPase and the reaction between ATP and rabbit muscle actomyosin (Gorog & Kovacs, 1970). The present results showed that responses to noradrenaline and especially Ca^{2+} recover after changing to indomethacin-free solution. No such recovery would be expected if indomethacin were acting solely to

inhibit prostaglandin synthesis, since kinetic studies using cell-free systems have shown indomethacin to be a 'competitive irreversible inhibitor' of cyclo-oxygenase (Flower, 1974). Further, indomethacin inhibits cyclo-oxygenase in rat tissues *in vivo* for at least 24 h (Fitzpatrick & Wynalda, 1976). Manku & Horrobin (1976) have also demonstrated that the inhibitory effect of indomethacin on vasoconstriction is readily reversed on changing to indomethacin-free solution. It was suggested that although this effect could be unrelated to prostaglandin synthesis inhibition, extrapolation from cell-free and *in vivo* systems to isolated preparations can be misleading because they do not take account of *de novo* enzyme synthesis and persistance of indomethacin respectively. Ideally, in *in vitro* experiments, a minimum concentration of indomethacin should be used to inhibit cyclo-oxygenase selectively. Unfortunately, this concentration has not yet been adequately established.

An attempt was made to clarify whether prostaglandin synthesis is necessary for the vasoconstrictor effect of Ca^{2+} by the use of a second cyclo-oxygenase inhibitor. 5,8,11,14-Eicosatetraynoic acid (ETA) was selected because it is chemically unrelated to indomethacin and appears to act as a false substrate on the catalytic site of cyclo-oxygenase where its action is rapid and irreversible (Flower, 1974). Even so, ETA inhibited responses to both noradrenaline and Ca^{2+} and like indomethacin, was more effective against noradrenaline. Previous results have shown that the inhibitory effect of ETA on noradrenaline-induced responses of rat mesenteric blood vessels is selective, since inhibition is overcome by adding PGE_2 to the perfusate (Coupar, 1980a). However, there is as yet no information in the literature concerning any potential non-selective antagonism of Ca^{2+} by ETA.

Exogenous prostaglandins were next added to the solutions containing the cyclo-oxygenase inhibitors to determine whether the inhibition of vasoconstrictor responses was due to prostaglandin deficiency. In this respect, PGE_2 was ineffective at restoring the response to Ca^{2+} depressed by indomethacin (62 μg/ml) or ETA (10 μg/ml). Again these results are in contrast to those of Manku & Horrobin (1976) who found that low concentrations of PGE_2 (5 ng/ml) reverse responses to Ca^{2+} depressed by 64 μg/ml of indomethacin.

Moreover, if PGE_2 is necessary to Ca^{2+}-induced responses, as Manku & Horrobin claim, Ca^{2+} may be expected to cause the release of the prostaglandin as does noradrenaline (Wolfe *et al.*, 1979; Coupar, 1980a). Although a small amount of PGE_2-like release was detected from the blood vessels, there was no concurrent increase in PGE_2 release when constriction was induced by Ca^{2+}. It is possible that another prostaglandin may have been released but was not detected by the bioassay tissue. For example, prostacyclin (PGI_2) spontaneously hydrolyses at physiological pH to 6-oxo-$PGF_{1\alpha}$ which is virtually inactive on the rat fundus strip (Whittle, Mugridge & Moncada, 1979). Although PGI_2 is released from rat mesenteric blood vessels (Horton, Pipili & Poyser, 1980) it is unlikely that it aids Ca^{2+}-induced constriction since it directly inhibits responses to noradrenaline and does not affect the response to extracellular Ca^{2+} (Ally, Barrette, Cunnane, Horrobin, Karmali, Karmazyn, Manku, Morgan & Nicolaou, 1978).

Apart from PGE_2, two other stable compounds were tested for ability to restore responses to Ca^{2+} in the presence of indomethacin. These were PGA_1, which has been shown to restore indomethacin-depressed responses to noradrenaline to above control level (Coupar & McLennan, 1978) and the stable epoxymethano analogue of PGH_2, U-46619.

Prostaglandin A_1 restored responses to Ca^{2+} in the presence of 62 μg/ml of indomethacin over the same concentration range (0.2–6 μg/ml) as shown previously to restore responses to noradrenaline depressed by only 25 μg/ml of indomethacin (Coupar & McLennan, 1978). It is likely that these effects of PGA_1 are pharmacological rather than physiological because of the relatively large doses needed and the doubt that PGA_1 is a naturally occurring prostaglandin. U-46619 also completely restored responses to Ca^{2+} but was more potent than PGA_1 and produced its effect over a wider concentration range. The differing results in the presence of PGE_2, A_1 and U-46619 indicate, perhaps surprisingly, that the high concentration of indomethacin used (62 μg/ml) does not cause general tissue depression. The mechanism by which PGA_1 reverses the responses to Ca^{2+} in the presence of indomethacin is not known. In a previous study, it was suggested that PGA_1 restores indomethacin-depressed responses to noradrenaline by acting on a different receptor from that acted on by PGE_1 and E_2 (Coupar & McLennan, 1978). Although there is no direct evidence, the mechanism by which U-46619 restores the response to Ca^{2+} in the presence of indomethacin may be related to its ability to decrease the amount or effect of cyclic AMP. The indirect evidence for this suggestion is that cyclic AMP is implicated as the intracellular mediator of vasodilatation (see Anderson, Nilsson, Wikberg, Johanson & Lundholm, 1975; Demesy-Waeldele & Stoclet, 1977; Gagnon, Regoli & Rioux, 1980). Further, the present results show that U-46619 reverses responses to Ca^{2+} depressed by papaverine, which is a phosphodiesterase inhibitor (Weiss, 1975). Finally, indomethacin at moderate concentrations, has been shown to inhibit phosphodiesterase (Flower, 1974). If the above proposal is correct, then the failure of U-46619 to reverse responses to Ca^{2+}

depressed by ETA, implies that ETA does not produce its effect by elevation of intracellular levels of cyclic AMP. Although U-46619 (200 ng/ml) alone caused a 1.6 fold potentiation of responses to Ca^{2+}, this small direct effect is insufficient to account for the full reversal of indomethacin- and papaverine-induced inhibition of Ca^{2+}.

Previous results have shown that U-46619 constricts rat mesenteric blood vessels perfused with Krebs-Henseleit solution containing the normal amount of Ca^{2+} (Coupar, 1980a). The present results therefore indicate that the prostanoid relies mainly on the presence of extracellular Ca^{2+} to produce its effect. The small rise in perfusion pressure (15 mmHg) produced by U-46619 (200 ng/ml) in Ca^{2+}-free depolarizing solution may result from mobilization or sequestration of a small intracellularly bound store of Ca^{2+}. It is becoming apparent that U-46619 mimics the effects of thromboxane A_2 (TXA_2) more closely than PGH_2. For example, U-46619 not only induces vasoconstriction but also stimulates platelet aggregation and constricts the airways (Beckmann & Leovey, 1976; Malmsten, 1976). Although PGH_2 also produces these effects, they are probably mediated by its conversion to TXA_2 (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980).

In conclusion, the results described in this paper do not support the hypothesis that prostaglandin synthesis is necessary to aid Ca^{2+}-induced vasoconstriction. PGE_2, although it might be necessary for the constrictor action of noradrenaline, is not released by Ca^{2+} and does not reverse the inhibition of Ca^{2+}-induced constriction caused by high concentrations of indomethacin or ETA. The results do not exclude the possibility that a prostaglandin might facilitate intracellular Ca^{2+} release triggered by the action of a vasoconstrictor.

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