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Release of prostaglandin E-like material from perfused mesenteric blood vessels of rabbits

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Infusions of noradrenaline (1–3 μg ml^{-1} min^{-1}) into the mesenteric vascular preparation of the rabbit caused a 2 to 5 fold rise in perfusion pressure and a release of prostaglandin E-like material (3·23 $\pm$ 0·65 (s.e.) ng PGE_2 equivalents ml^{-1}). Indomethacin (3 μg ml^{-1}) prevented whereas arachidonic acid (0·2 μg ml^{-1}) augmented, the noradrenaline-evoked release of a prostaglandin E-like material. The walls of arterioles or precapillary vessels are the proposed site of prostaglandin generation.

Recently it has been demonstrated that prostaglandin E-like material (PGE) appears in the circulation during the intravenous infusion of noradrenaline into cats (Gryglewski & Ocetkiewicz, 1974). PGE is also released by constricted perfused rabbit ear vessels (Gryglewski & Korbut, 1975). Experimental evidence for the suggestion that PGE is generated by the constricting vascular wall (Hedqvist, 1972; Aiken, 1974) has thus been obtained, but it is not clear what segments of the vascular tree are responsible for PGE release.

We report here that noradrenaline-evoked vasoconstriction does not liberate PGE from perfused or superfused rabbit arteries unless the small distal vessels (probably arterioles or precapillary vessels) are left in the vascular preparation.

MATERIALS AND METHODS

Rabbits of either sex, 2–3 kg, were killed by a blow on the neck. The superior mesenteric artery was cannulated and removed together with all branches supporting the ileum at a length of 80–100 cm. The vascular mesenteric preparation was placed in a heated (37°) chamber and perfused with Krebs bicarbonate solution (37°), bubbled with 5% CO_2 in oxygen. The perfusion fluid was pumped at a rate of 2·5 ml min^{-1} to maintain the perfusion pressure at a level of 5–10 mm Hg as measured by a mercury manometer. The Krebs solution had the following composition (mM): NaCl 118, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ $7H_2O$ 1·17, $CaCl_2 6H_2O$ 2·5, $NaHCO_3$ 25·0 and glucose 8·4. In some experiments the left renal artery was perfused after cutting it distally at its entrance to the kidney hilum. Some studies were made on spirally-cut strips of superfused rabbit thoracic aorta.

The effluent from vascular preparations superfused various assay tissues in turn (Vane, 1964): these included a rat stomach strip (RSS), a rat colon (RC), and sometimes a rabbit aorta strip (RbA). Their movements were registered mechanically by auxotonic levers (Paton, 1957) with a magnification of 8 and an initial load of 2–3 g.

The specificity of assay organs for prostaglandins was increased by infusing the following antagonists directly over the assay organs: atropine 0·1, methysergide 0·1, propranolol 2, phenoxybenzamine 0·5, antazoline 0·1 and indomethacin 3 (μg ml^{-1}), (Gilmore, Vane & Wyllie, 1968). Indomethacin or phenoxybenzamine could be

infused separately into the vascular preparation. Intra-arterial infusions of noradrenaline (1–3 μg ml^{-1} min^{-1}) were used to produce vasoconstriction. Since noradrenaline relaxed the assay organs, it was removed from the effluent by an aluminium oxide column (Korbut, 1975) incorporated at the top of the cascade system. The calibration doses of PGE_2 and $PGF_{2\alpha}$ were administered through the aluminium oxide column.

RESULTS

Infusions of noradrenaline at a rate 1–3 μg ml^{-1} min^{-1} into the rabbit isolated renal artery increased perfusion pressure by 200–300%, but no detectable amounts (>0·25 ng ml^{-1}) of prostaglandin E-like material (PGE) appeared in the effluent, nor did noradrenaline-induced maximal contraction of the spirally-cut thoracic aorta strip release any PGE. Infusion of noradrenaline (1–3 μg ml^{-1} min^{-1} during 5 min) into 20 mesenteric vascular preparations raised the perfusion pressure by 200–500% and released PGE (3·23 $\pm$ 0·65 (s.e.) ng PGE_2 equivalents ml^{-1}). There was no release of rabbit aorta contracting substance (RCS).

In some preparations small branches of the arch of the mesenteric artery were tied and partially cut away. In these preparations no PGE was released into the perfusate in spite of a distinct pressor effect of noradrenaline.

The PGE-like character of the released material was confirmed by its differential contractile potency on rat stomach strip and colon (Fig. 1) and by the reversible prevention of its release with indomethacin (Fig. 1). Noradrenaline-induced generation of PGE and the increase in perfusion pressure were both prevented by phenoxybenzamine (0·5 μg ml^{-1} min^{-1}). Repeated half-hourly infusions of noradrenaline into mesenteric preparations caused progressively smaller release of PGE (Fig. 1). The relation between the induced PGE release and the pressor response was not uniform, but tended to be inversely related. Thus the second and third pressor responses were usually higher than the first (Fig. 1).

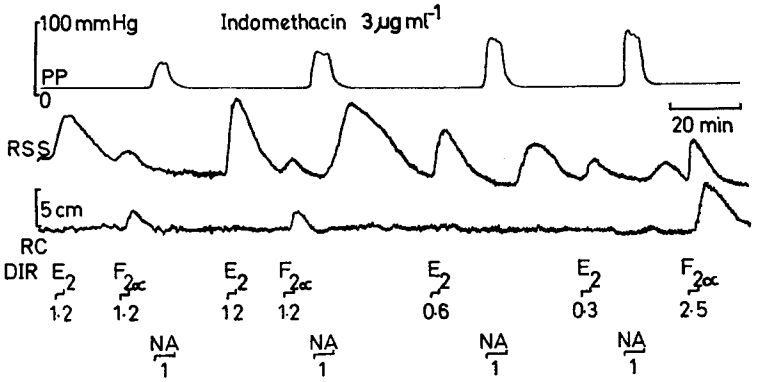

FIG. 1. Noradrenaline (NA)-evoked release of prostaglandin E-like material (PGE) from a vascular mesenteric preparation of the rabbit. A rat stomach strip (RSS) and a rat colon (RC) were superfused in cascade (see Methods) with the perfusate from a mesenteric preparation (2·5 ml min^{-1}). An aluminium oxide column was placed between the vascular preparation and the assay organs. Sensitivity of the assay organs to PGE_2 and $PGF_{2\alpha}$ was calibrated by direct (DIR) infusions of prostaglandins (ng ml^{-1}) through the aluminium oxide column. NA (1 μg ml^{-1} min^{-1}) was infused into the mesenteric artery. In the presence of indomethacin (3 μg ml^{-1}) there was no PGE-release during the increase in perfusion pressure (PP) induced by NA 1 h after indomethacin was removed 3 consecutive infusions of NA released PGE. The negative correlation between the amounts of PGE released and the height of pressor response can be seen, except for the first infusion of NA when indomethacin was present in the perfusing fluid.

The noradrenaline-evoked release of PGE was augmented in the presence of arachidonic acid (0·2 μg ml^{-1}) (Fig. 2). Concentrations of arachidonic acid higher than 0·5 μg ml^{-1} depressed the vascular response to noradrenaline and in most cases also decreased the sensitivity of the assay organs to prostaglandins.

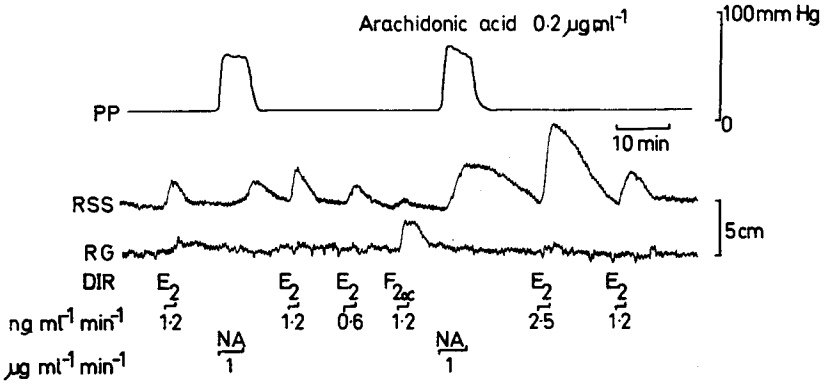

FIG. 2. In the presence of noradrenaline (NA) and arachidonic acid (0·2 μg ml^{-1}) into a perfused vascular mesenteric preparation the increase of the perfusion pressure (PP) by NA (1 μg ml^{-1} min^{-1}) showed tachyphylaxis and at the same time an augmented amount of PGE appeared in perfusate (RSS contraction). Other explanations as in Fig. 1.

Tachyphylactic pressor response to the noradrenaline infusion was observed when arachidonic acid was present in the perfusate (Fig. 2). Indomethacin (3 μg ml^{-1}), however, abolished acute tolerance to noradrenaline, although the height of the pressor response to noradrenaline was sometimes reduced (Fig. 1).

In indomethacin-pretreated vascular preparations the pressor response to noradrenaline (1–3 μg ml^{-1} min^{-1}) was not influenced by a simultaneous infusion of PGE_2 1–40 ng ml^{-1} min^{-1} (4 experiments).

DISCUSSION

Horrobin, Manku & others (1974), reported that in rat mesenteric preparations indomethacin (5 μg ml^{-1}) or aspirin (5–20 μg ml^{-1}) inhibited the pressor response to noradrenaline (0·1 ng), while infusions of PGE_2 (1–1000 pg ml^{-1}) partially restored the responsiveness. However in our experiments PGE_2 (1–40 ng ml^{-1}) did not influence the pressor response to noradrenaline in the indomethacin-pretreated mesenteric vascular bed of rabbits. Nor did it alter the pressor response to either noradrenaline or angiotensin in the perfused dog hindpaw (Kadowitz, Sweet & Brody, 1971), but PGE_1 inhibits the vasoconstrictor action of noradrenaline in the mesenteric vascular smooth muscle of the rabbit (Strong & Chandler, 1972) and in the dog hindpaw (Kadowitz & others, 1971).

Horrobin & others (1974) have proposed that endogenous PGE is necessary for combination of noradrenaline with its receptor site to initiate vasoconstriction. This explanation contradicts the concept that endogenous PGE counteracts the excessive vasoconstriction produced by catecholamines (Hedqvist, 1972) or by angiotensin (Aiken, 1974). The antagonistic action of endogenously generated PGE on the noradrenaline-evoked vasoconstriction has been demonstrated experimentally in anaesthetized cats (Gryglewski & Ocetkiewicz, 1974) and in perfused rabbit ear preparations (Gryglewski & Korbut, 1975).

In this study we have also found that the amounts of PGE released from the constricted mesenteric vascular bed are inversely proportional to the height of pressor response to noradrenaline or to the degree of acute tolerance to it. A possible explanation of the discrepancy between the results of Horrobin & others (1974) and ours may be either the difference in noradrenaline dosage or the difference in the species of animal.

We had to use 1–3 μg ml^{-1} noradrenaline to obtain measurable release of PGE (>0·25 ng ml^{-1}), while Horrobin & others (1974) used doses at least 10 000 times lower than these. In view of the evidence presented by Horrobin & others (1974) it is possible that minute amounts of noradrenaline release less PGE than we can detect, and that PGE may enhance the vasoconstrictor effect of noradrenaline in the rat mesenteric preparation. Our experiments produced direct evidence that microgram concentrations of noradrenaline release a few nanograms of PGE in the rabbit mesenteric vessels. At these concentrations endogenous PGE usually either diminished the noradrenaline-induced pressor response or evoked tachyphylaxis to noradrenaline. Sometimes no effect on the pressor response was observed, but potentiation never occurred.

Recently Mullane, Armstrong & McGiff (1975) have demonstrated that PGE_2 (10–100 ng ml^{-1}) constricts renal blood vessels in normotensive and genetically hypertensive rats of the New Zealand strain. In these animals a potentiation by PGE_2 of renal vascular sensitivity to pressor effects of noradrenaline, angiotensin II and 5-hydroxytryptamine has also been observed, whereas indomethacin (3–6 μg ml^{-1}) diminished pressor response to those vasoconstrictors. Thus the difference in the species of animal is the most probable explanation for the discrepancy between the results of Horrobin & others (1974) and ours. Unlike in rabbits, dogs and cats, in rats PGE seems to potentiate vascular sensitivity to noradrenaline and to other pressor hormones.

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