The differential response of a novel bioassay tissue, the rabbit transverse stomach-strip, to prostacyclin (PGI_2) and other prostaglandins

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The usefulness of various isolated tissues in the preliminary identification of prostaglandins (PGs) by parallel bioassay depends on their sensitivity and discrimination. We now describe a novel tissue, the transverse stomach muscle of the rabbit, which in conjunction with the rabbit coeliac artery (Bunting, Moncada & Vane, 1976) and the rat stomach strip (Vane, 1957), can distinguish between the majority of the primary prostaglandins.

A transverse segment (approximately 30 mm × 10 mm) of the middle region of the rabbit stomach was excised and the gastric mucosa was stripped off. The remaining muscle layer was cut along its long axis to form a strip and suspended (under 1 g load) for superfusion in cascade (Vane, 1964). Strips of rabbit-coeliac artery (RbCA) and rat stomach strip (RSS) were prepared as previously described. The tissues were superfused with oxygenated Krebs solution (5 ml/min; 37°C), usually containing indomethacin (2 μg/ml) and a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) to increase the specificity of the tissues.

This transverse segment of rabbit stomach (RbSS) responded by contraction or relaxation to the various prostaglandins (Figure 1), unlike longitudinal segments which responded by contraction only. The mean threshold for relaxation was 1 ng for PGE_1 or E_2, 5 ng for PGD_2 and 10 ng for PGA_2 (from 6 experiments), and the muscle recovered basal tone within 5–8 minutes. Thus, unlike many of the other bioassay tissues, the RbSS detected both PGA_2 and PGD_2 in low concentrations. PGA_2, unlike PGD_2 relaxed RbCA and both, unlike PGE_2 had no effect on RSS in comparable doses (Figure 1). Prostacyclin (PGI_2) caused a rapid and short-lasting contraction of RbSS, (threshold, 5–10 ng). $PGF_{2\alpha}$ also contracted RbSS (threshold, 20 ng) but had a different profile of activity on the other tissues (Figure 1). The breakdown product of prostacyclin, 6-oxo-$PGF_{1\alpha}$ (250 ng), had little effect on the tissues.

The endoperoxide PGH_2 contracted RbSS, while thromboxane A_2, generated by incubating PGH_2 with horse platelet microsomes (Bunting *et al.*, 1976) caused a small contraction of RbSS, but strongly contracted RbCA. Infusion of arachidonic acid (4 μg/ml) caused a slow contraction of RbSS which was abolished by indomethacin (2 μg/ml).

Transverse segments of stomach muscle from rat or guinea-pig were more difficult to prepare and the responses less reproducible. These observations indicate that RbSS is a useful tissue for the identification of known arachidonic acid metabolites and for the detection of possible new products.

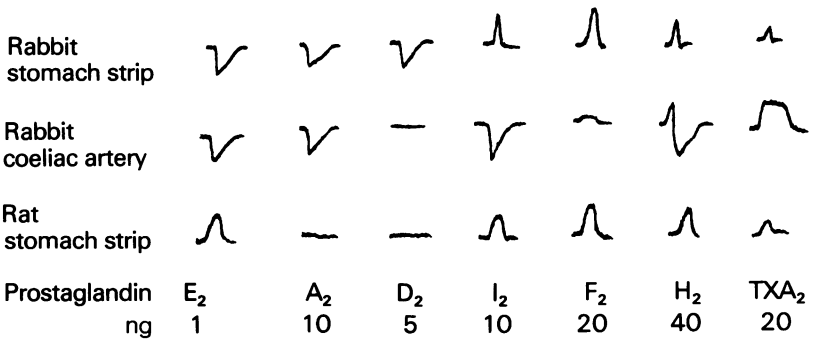

Figure 1 Profile of responses of the isolated bioassay tissues, the rabbit stomach strip, the rabbit coeliac artery and the rat stomach strip to several arachidonic acid metabolites.

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Effect of divalent cations on lysosomal enzyme release from macrophages

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Macrophages constitute the main cell type in chronic inflammatory lesions (Davies, 1976) and may secrete a variety of pro-inflammatory products including lysosomal enzymes (LE), lysosyme, plasminogen activator, collagenase and elastase (Unanue, 1976). Divalent cations play a central role in stimulus-secretion coupling in other inflammatory cells (Henson, 1974) but their role in macrophage secretion is not known. In the present study, we have investigated the effect of divalent cations on LE release from macrophages, using zymosan and the divalent cation ionophore A23187 as stimuli.

Thioglycollate-stimulated mouse peritoneal macrophages were prepared as previously described (Gordon, MacIntyre & McMillan, 1977). Release of LE (β-N-acetyl-glucosaminidase) was determined fluorometrically, by a modification of the method described for platelet LE release by Gordon (1975). As an indicator of cell lysis, release of the cytoplasmic enzyme lactate dehydrogenase (LDH) was determined photometrically, by a modification of the method of Wroblewski & La Due (1955). Zymosan suspensions were prepared as described by Weissmann, Dukor & Zurier (1971) and ionophore A23187 was dissolved in dimethylsulphoxide. To determine the role of extracellular divalent cations, macrophages were incubated with zymosan or A23187 in calcium and magnesium-free Hanks buffered salt solution and known amounts of calcium and magnesium were added.

Incubation of macrophages for 4 h at 37°C with zymosan (25–250 μg/ml) resulted in selective release of LE without leakage of LDH. Zymosan-induced release of LE occurred in the absence of extracellular divalent cations. However, release was enhanced by magnesium (0.3–5 mM) and inhibited by calcium (0.3–5 mM). In the presence of equimolar calcium and magnesium, LE release was similar to that produced in the absence of both.

Incubation of macrophages with A23187 (0.1–30 μM) for 2 h at 37°C led to release of both LE and LDH. Release occurred only when divalent cations were present extracellularly. Calcium was more potent than magnesium at facilitating release and the effect of the two ions was additive.

A23187-induced release of LE from macrophages, which occurs only in the presence of extracellular divalent cations, is essentially a lytic process. This is in marked contrast to the selective secretion induced by A23187 in other cell types (Foreman, Mongar & Gomperts, 1973; Feinman & Detwiler, 1974). Zymosan-induced secretion of macrophage LE did not require extracellular calcium or magnesium although secretion was enhanced by magnesium and inhibited by calcium. The influence of divalent cations on zymosan-induced LE secretion may be related to an effect on phagocytosis which is dependent on magnesium but not calcium (Lay & Nussenzweig, 1968; Henson, 1969). Further studies are in progress to investigate the role played by intracellular cations in mediating secretion of LE from macrophages.

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