The release of thromboxane B_2 by rabbit peritoneal polymorphonuclear leucocytes

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Polymorphonuclear leucocytes (PMNs) elicited in the rabbit peritoneal cavity by the injection of glycogen have been reported to release prostaglandins of the E and F series during phagocytosis (Higgs, McCall & Youlten, 1975). Homogenized preparations of the same cell types convert prostaglandin endoperoxides to a substance possessing thromboxane A_2-like activity (Higgs, Bunting, Moncada & Vane, 1976) and human peripheral PMNs produce thromboxane B_2 as detected by a radioimmunoassay method (Goldstein, Malmsten, Kaplan, Jindahl, Samuelsson & Weissmann, 1977). In the present work we have used a more specific gas–liquid chromatography–mass spectrometry technique to examine the release of prostaglandins and thromboxanes from rabbot peritoneal PMNs.

The PMNs were elicited by the intraperitoneal injection of 100 ml of 0.1% (w/v) of oyster glycogen into New Zealand white rabbits (2.5 kg) and harvested according to the directions of Hirsch & Church (1960). After washing, the cells were suspended in Medium 199 containing 30 mM HEPES buffer, pH 7.4, to give a final concentration of 10^7 PMNs ml^{-1}. Aliquots (10 ml) were incubated at 37°C for 2 h in either the absence or the presence of 1 mg ml^{-1} of rabbit serum opsonized zymosan. The supernatant, prepared by removing the cells by centrifugation, was acidified to pH 3.5 and extracted three times with diethyl ether. The ether was removed from the combined extracts under reduced pressure, the residue dissolved in 0.1 ml methanol and reacted with 1 ml of diazomethane for 1 minute. After repetition of the methylation stage, the sample was transferred to a reacti-vial, the solvent removed under nitrogen and allowed to react with 10 µl of methoxyamine HCl in pyridine (2.5 mg/ml) for 16 h at 20°C and then for 30 min at 60°C. Removal of the solvent under nitrogen was followed by exposure to bistrimethylsilyl trifluoroacetamide for 15 min at 60°C and chromatographed at 230°C using a 1% SE 30 Chromosorb W (80–100 mesh) column (1.5 m × 0.4 cm) in a Pye 104 oven interfaced to a VG Micromass 16B mass spectrometer (interface heater, 250°C; source heater, 240°C; spectra obtained at 20 eV, 100 µA trap current).

The major end product of the prostaglandin cyclooxygenase system found in the present work was thromboxane B_2 (characteristic fragment ions m/e 301, 211, 191, 174 and 173). This substance was consistently present in the supernatants obtained from the PMNs incubated with or without opsonized zymosan. Its release, together with that of β-glucuronidase, was increased in the zymosan experiments. Trace amounts of $PGF_{2\alpha}$ were occasionally detected but no evidence of the presence of other prostaglandins was obtained. The work is being extended to include the use of deuterated thromboxane B_2 as an internal standard in order to provide quantitative data.

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