Generation of thromboxane A_2-like activity from prostaglandin endoperoxides by polymorphonuclear leukocyte homogenates

S. BUNTING, G.A. HIGGS, S. MONCADA & J.R. VANE

The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS.

Rabbit aorta contracting substance (RCS) released from isolated perfused lungs of guinea pigs during anaphylaxis (Piper & Vane, 1969) is now thought to be a mixture of thromboxane A_2 (TXA_2) and prostaglandin endoperoxides (PGG_2, PGH_2; Hamberg, Svensson & Samuelsson, 1975; Bunting, Moncada & Vane, 1976). TXA_2 is generated enzymically from PGG_2 or PGH_2 by horse or human platelet microsomes (Bunting, Moncada, Needleman & Vane, 1975). TXA_2 is distinguished from endoperoxides by its shorter half life and its increased potency in contracting the rabbit aorta; moreover, while prostaglandin endoperoxides relax the mesenteric and coeliac arteries of the rabbit, TXA_2 is a powerful constrictor of both (Bunting, Moncada & Vane, 1976).

Rabbit peritoneal polymorphs (PMNs) release prostaglanin during phagocytosis (Higgs, McCall & Youlten, 1975). We have now shown that homogenates of these cells contain an enzyme which converts PG endoperoxides into a substance with increased RCS activity which contracts the rabbit coeliac artery and has a similar half life to TXA_2.

PMN leukocyte suspensions obtained by the method of Hirsch & Church (1960) were centrifuged (50 *g* for 10 min), washed once with 0.9% saline and resuspended in 5 ml Krebs bicarbonate solution at a concentration of $4-18 \times 10^7$ cells/ml. The cells were disrupted by freezing and thawing five times and homogenized in a glass homogenizer. The homogenates were then centrifuged at 100,000 *g* for 1 h and resuspended in 2 ml 100 mM Tris buffer (pH 7.5) prior to incubation with PGG_2 or PGH_2 (1 μg/ml). The biological activity of the incubation mixtures was assayed on spirally cut strips of rabbit aorta and rabbit coeliac artery, superfused at 10 ml/min with Krebs bicarbonate containing a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) plus indomethacin (2 μg/ml). Changes in length of the assay tissues were detected by Harvard smooth muscle transducers and displayed on a Watanabe pen recorder.

Homogenates of PMNs were incubated with PGG_2 or PGH_2 for 2 min at 0°C to test for thromboxane synthetase activity. None was detected in resting PMNs but when they were incubated with killed bacteria (Pertussis vaccine, Burroughs Wellcome) for 1 h at 37°C prior to homogenization, increased RCS activity was generated and the coeliac artery contracted. This conversion was prevented by boiling the homogenate prior to incubation, or by addition of benzydamine (200 μg/ml) a drug which inhibits the formation of TXA_2 by horse platelet microsomes (Moncada, Needleman, Bunting & Vane, unpublished) but not indomethacin (10 μg/ml). Incubation mixtures at neutral pH and 0°C were rapidly extracted with dry ether; the ether was evaporated under nitrogen and the residue taken up in buffer. Extracts contained RCS activity with a half life of 10–11 min at 0°C, 1–2 min at room temperature and less than 1 min at 37°C; values similar to those described for TXA_2.

These results show that homogenates of phagocytosing PMNs generate thromboxane A_2-like activity. Thromboxane B_2, the metabolite of TXA_2 is chemotactic (Boot, Dawson & Kitchen, 1976); thus the production of thromboxanes by PMNs during phagocytosis could contribute to the inflammatory process.

References

BOOT, J.R., DAWSON, W. & KITCHEN, E.A. (1976). The chemotactic activity of thromboxane B_2: a possible role in inflammation. *J. Physiol. (Lond.)*, in press

BUNTING, S., MONCADA, S., NEEDLEMAN, P. & VANE, J.R. (1975). Formation of prostaglandin endoperoxides and rabbit aorta contracting substance (RCS) by coupling two enzyme systems. *Br. J. Pharmac.*, **56**, 344P–345P.

BUNTING, S., MONCADA, S. & VANE, J.R. (1976). The effects of prostaglandin endoperoxides and thromboxane A_2 on strips of rabbit coeliac artery and certain other smooth muscle preparations. *Br. J. Pharmac.*, in press.

GILMORE, N., VANE, J.R. & WYLLIE, H.J. (1968). Prostaglandins released by the spleen. *Nature (Lond.)*, **218**, 1135–1140.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1975). Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. Nat. Acad. Sci., U.S.A.*, **72**, 2994–2998.

HIGGS, G.A., McCALL, E. & YOULTEN, L.J.F. (1975). A chemotactic role for prostaglandins released from polymorphonuclear leukocytes during phagocytosis. *Br. J. Pharmac.*, **53**, 539–546.

HIRSCH, J.G. & CHURCH, A.B. (1960). Studies of phagocytosis of group A streptococci by polymorphonuclear leukocytes *in vitro*. *J. exp. Med.*, **III**, 309–322.

PIPER, P.J. & VANE, J.R. (1969). The release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature (Lond.)*, **223**, 29–35.