A convenient method for isolation and bioassay of prostaglandins E_1, E_2, F_2 and D_2

J.P. DENTON, P. MARPLES & A.L. WILLIS

Department of Pharmacology, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY.

We routinely assay blood and tissue prostaglandins (PG's) and our method of PG isolation and bioassay is described and demonstrated here.

Samples (2.5 ml) of platelet-rich plasma (PRP) or tissue (<2 g) were transferred into polypropylene vials and snap-frozen (< −60°C) and stored (−20°C) for less than 5 weeks before analysis. At 1–2 days prior to extraction, tissue samples (skin, lung, etc.) were powdered under liquid nitrogen in a freezer mill (Spex; Metuchen, N.J.). The powdered material was stored at −80°C.

For extraction, the powdered tissue or frozen PRP was transferred into screw-top polyproplylene tubes, each containing ~0.1 μCi (~250 pg) of [^{3}H]-labelled PGE_1, PGE_2, and $PGF_{2\alpha}$ (New England Nuclear) and thawed into 5 ml (for PRP) or 10 ml (for ground tissue) of saturated NaCl solution. This mixture was immediately acidified to pH 2.8 with HCl and 15 ml of ethyl acetate added. The aqueous layer was frozen and the ethyl acetate layer filtered through phase-separating paper (Whatman IPS). After further extraction with ethyl acetate and drying the combined extract over anhydrous Na_2SO_4, the solution was reduced in polarity with excess petroleum ether and subjected to a rapid silicic acid column procedure (Willis & Weiss, 1973). This separates the PGs (E, F and D) from less polar and more polar lipid contaminants.

The PG-containing fraction (methanol:ethyl-acetate, 1.5:8.5) was then evaporated to dryness, dissolved in 200 μl of the methanolic ethyl acetate and subjected to thin layer chromatography (TLC) using precoated silica-gel plates, impregnated with silver nitrate. The plates were developed in the AII solvent system of Gréen & Samuelsson (1964) and position of the PG's located by visualization of authentic markers and radiochromatogram scanning. Appropriate zones (E_1; $F_{2\alpha}$; $E_2 + D_2$) of the chromatographed extract were scraped off, soaked in saline and extracted (at pH 3) into ethyl acetate (Willis, 1970). After evaporating the ethyl acetate to dryness, material from the PGE_1 and $PGF_{2\alpha}$ zones was stored at −20°C until dissolved in 0.1–1 ml of tyrode's solution and submitted to bioassay.

Part of the extracted material from the $PGE_2 + D_2$ zone was again subjected to TLC on silica gel using the solvent system of Sun (1977) which separated PGD_2 (Rf 0.6) from PGE_2 (Rf 0.24). After scraping, eluting and extracting E_2, D_2 and blank zones, the dried extracts were stored (−20°C) until assay.

The extracts were each dissolved in ~100 μl of saline, and then material from PGD_2 (and sometimes also PGE_1) zones were assayed in terms of authentic standard for ability to inhibit ADP-induced aggregation of platelets treated with aspirin (20 μg/ml); this method is somewhat similar to that described by Oelz, Oelz, Knapp, Sweetman & Oates (1977).

PG's E_2, E_1 and $F_{2\alpha}$ were routinely assayed using superfused rat stomach strip (Vane, 1957) using aspirin (20 μg/ml) and a mixture of antagonists (Gilmore, Vane & Wyllie, 1968) to enhance selective sensitivity of the tissue.

We acknowledge Miss E. Morris for technical help and Professor E.W. Salzman for a gift of PGD_2.

References

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

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX Thin layer chromatography of prostaglandins. *J. Lipid Res.*, **5**, 117–120.

OELZ, O., OELZ, R., KNAPP, H.R., SWEETMAN, B.J. & OATES, J.A. (1977). Biosynthesis of prostaglandin D_2. 1. Formation of prostaglandin D_2 by human platelets. *Prostaglandins*, **13**, 225–234.

SUN, F.F. (1977). Biosynthesis of thromboxanes in human platelets I. Characterization and assay of thromboxane synthetase. *Biochem. Biophys. Res. Commun.*, **74**, 1432–1440.

WILLIS, A.L. (1970). Simplified thin layer chromatography of prostaglandins in biological extracts. *Br. J. Pharmac.*, **40**, 583P.

WILLIS, A.L. & WEISS, H.J. (1973). A congenital defect in platelet prostaglandin production associated with impaired haemostasis in storage pool disease. *Prostaglandins*, **4**, 783–794.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxy-tryptamine. *Br. J. Pharmac.*, **12**, 344–349.