Action of a series of non-steroid and steroid anti-inflammatory drugs on prostaglandin synthesis by the microsomal fraction of rat skin

M.W. GREAVES, W.P. KINGSTON* & KATHLEEN PRETTY

University Department of Dermatology, Newcastle upon Tyne

We have obtained a prostaglandin synthetase-rich microsomal fraction from skin and we report the action of non-steroid and steroid anti-inflammatory drugs on prostaglandin synthesis by this preparation.

Freshly excised rat skin was homogenized in tris buffer pH 8.2 (0.2 M). The microsomal pellet was obtained by differential ultracentrifugation and indentified by electron microscopy and measurement of the marker enzyme glucose-6-phosphatase. Prostaglandin biosynthesis by the microsomal fraction was measured by both the radiochemical method and the adrenochrome spectrophotometric method of Takeguchi and Sih (1972).

For the radiochemical method the enzyme preparation was incubated for 20 min at 37° C with the following: arachidonic 0.2 mM: ^{3}H-arachidonic acid, 0.5 μCi; glutathione 0.65 mM; hydroquinone 0.275 mM and bovine serum albumin 10 mg. Prostaglandins E_2 and $F_{2\alpha}$ were extracted from the incubation mixture and identified by the thin-layer chromatographic method of Greaves & McDonald-Gibson (1972a). The reaction mixture in the adrenochrome assay contained arachidonic acid 0.2 mM, adrenaline tartrate 1.0 mM, tris buffer 8.2 (0.2 M). These reagents were pre-incubated for 5 min at 37° C, the reaction being initiated by addition of the pre-incubated mixture to the enzyme preparation. The change in absorbency at 480 nm was followed for 5 minutes. Control reactions with trichloracetic acid inactivated enzyme preparations were carried out in all experiments. The results are shown in Table 1. Inhibition was dose related and the results for the two assay methods agreed closely. Indomethacin, flufenamic acid and mefenamic acid were the most potent inhibitors of synthesis, although the concentrations required for inhibition were high (Vane, 1971; Ziboh, 1973). Five potent corticosteroid agents did not inhibit synthesis over a wide dose range. The corticosteroid results contrast with findings of an earlier study, using a crude skin homogenate as a source of prostaglandin synthetase activity, in which dose-related inhibition by fluocinolone acetonide was demonstrated (Greaves & McDonald-Gibson, 1972b). The results could be explained by the presence in the homogenates, but not in the microsomal fraction, of a factor which facilitates or potentiates an inhibitory action of corticosteroids.

TABLE 1 Inhibition of prostaglandin synthetase by anti-inflammatory drugs

	ID 50 (mM)	
Drug	*Adrenochrome Spectrophoto-metric assay*	*Radiochemical assay*
Indomethacin	0.75	0.61
Mefanamic	1.5	1.025
Flufenamic acid	1.8	1.27
Naproxen	9.0	*
Ibuprofen	13.5	*
Aspirin	14.0	10.05

* Not assayed

Paracetamol, Hydrocortisone, Dexamethasone, Betamethasone, Triamcinolone acetonide, Fluocinolone acetonide did not inhibit synthesis measured by either assay method over a wide dose range.

References

GREAVES, M.W. & McDONALD-GIBSON, W.J. (1972a). Extraction of prostaglandin-like activity from whole human blood. *Life Sci.*, **11**, 73-81.

GREAVES, M.W. & McDONALD-GIBSON, W.J. (1972b). Prostaglandin biosynthesis by human skin and its inhibition by corticosteroids. *Br. J. Pharmac.*, **46**, 172-175.

TAKEGUCHI, C. & SIH, C.J. (1972). A rapid spectrophotometric assay for prostaglandin synthetase: application to the study of non-steroidal anti-inflammatory agents. *Prostaglandins*, **2**, 169-184.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.*, **231**, 232-235.

ZIBOH, V.A. (1973). Biosynthesis of prostaglandin E_2 in human skin: subcellular localization and inhibition by unsaturated fatty acids and anti-inflammatory drugs. *J. Lipid Res.*, **14**, 377-384.