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# SULPHASALAZINE IS A POTENT INHIBITOR OF PROSTAGLANDIN 15-HYDROXYDEHYDROGENASE: POSSIBLE BASIS FOR THERAPEUTIC ACTION IN ULCERATIVE COLITIS

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Sulphasalazine is a potent and selective inhibitor *in vitro* of prostaglandin 15-hydroxydehydrogenase in rabbit colon ($ID_{50}$ = 50 μM) and in several other organs of different species, but does not inhibit prostaglandin Δ-13 reductase or microsomal prostaglandin synthesis from arachidonic acid. It is suggested that this action may underly the therapeutic usefulness of sulphasalazine in ulcerative colitis for the prevention of relapse.

**Introduction** Sulphasalazine has been used for over 30 years in the treatment of ulcerative colitis (Goldman & Peppercorn, 1975; Eastwood & Das, 1975) but the mechanism of action of this important drug remains unknown. Long-term maintenance therapy with sulphasalazine (0.5 to 4.0 g/day, orally) has been shown to be effective for preventing relapse after ulcer healing (Misiewicz, Lennard-Jones, Connell, Baron & Avery Jones, 1965) whereas anti-inflammatory steroids are more effective for treatment of an acute colitic attack (Watkinson, 1976). Ulcerative colitis is a chronic inflammatory disease of the mucous membrane of the large bowel, and is characterized clinically by recurrent attacks of bloody diarrhoea and pain, and pathologically by the presence of shallow ulcerous lesions in the colon and rectum (Goligher, de Dombal, Watts & Watkinson, 1968). Since there is evidence that prostaglandin deficiency may lead to gastric or duodenal ulceration (Robert, 1976) and, conversely, that excessive prostaglandin production may cause certain types of diarrhoea (Robert, 1976; Bennett, 1976), we have investigated the possible effect of sulphasalazine on prostaglandin synthesis and inactivation in the rat and rabbit. Our results show that the drug is a potent and selective inhibitor of prostaglandin 15-hydroxydehydrogenase (15-PGDH).

**Methods** Cell-free 100,000 *g* supernatants and pellets were prepared at 4°C from tissues homogenized in 4 vol 50 mM phosphate buffer pH 7.4 containing 1 mM cysteine and disodium edetate (EDTA). Prostaglandin metabolism was assayed radiochemically in the supernatants in incubations containing 10 μg/ml prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 5 mM $NAD^+$ (for 15-PGDH assay) or 10 μg/ml 15-keto $PGF_{2\alpha}$ and 5 mM NADH (for prostaglandin Δ-13 reductase assay) which were extracted after incubation at 37°C. Each incubation also contained 0.1 μCi tritiated $PGF_{2\alpha}$ (specific activity 81.6 Ci/mol) labelled at the 9-β position. The extracts were chromatographed on plastic-backed silica gel-coated t.l.c. sheets in ethyl acetate/acetone/glacial acetic acid (90:10:1, v/v). Metabolism was measured by comparison of the counts recovered in those zones of the chromatogram corresponding to substrate and $PGF_{2\alpha}$ metabolites located by parallel chromatography of reference standards (see Hoult & Moore, 1977). For synthesis experiments, the 100,000 *g* microsomal pellet was resuspended in boiled high-speed supernatant, and incubated at 37°C with 10 μg/ml arachidonic acid together with 3 mM reduced glutathione and 0.5 mM hydroquinone as cofactors. The incubations were extracted and, after reconstitution in a Krebs solution, were assayed biologically on the rat fundus strip preparation by a bracket assay with $PGE_2$ as reference standard (see Hoult & Moore, 1977).

**Results** Rabbit colon 100,000 *g* supernatants rapidly metabolize $PGF_{2\alpha}$ as this organ possesses a high content of 15-PGDH. The major product formed is 15-keto $PGF_{2\alpha}$; relatively little $PGF_{2\alpha}$ is converted to the second 'conventional pathway' prostaglandin metabolite, 13,14-dihydro-15-keto $PGF_{2\alpha}$, because rabbit colon is deficient in prostaglandin Δ-13 reductase (Moore & Hoult, 1978). Figure 1 shows that sulphasalazine is a powerful inhibitor of $PGF_{2\alpha}$ metabolism in rabbit colon supernatants, with an $ID_{50}$ of 50 μM, but has little effect on prostaglandin synthesis at concentrations up to 1 mM. By contrast, indomethacin is a much more powerful inhibitor of prostaglandin synthesis ($ID_{50}$ = 4.2 μM) although it also inhibits prostaglandin metabolism at higher concentrations ($ID_{50}$ = 250 μM). We have also found that sulphasalazine at 50 μM inhibits *in vitro* $PGF_{2\alpha}$ metabolism in a number of other organs and species: rat colon (43.9 ± 6.5% inhibition), rat kidney (53.3 ± 7.5%), rat lung (53.6 ± 5.7%), chick kidney (46.2 ± 1.9%), chick lung (58.2 ± 4.6%), rabbit lung (48.2 ± 4.6%), and rabbit kidney (30.7 ± 2.1%) (values are mean ± s.e. mean of 4 to 6 experiments for each

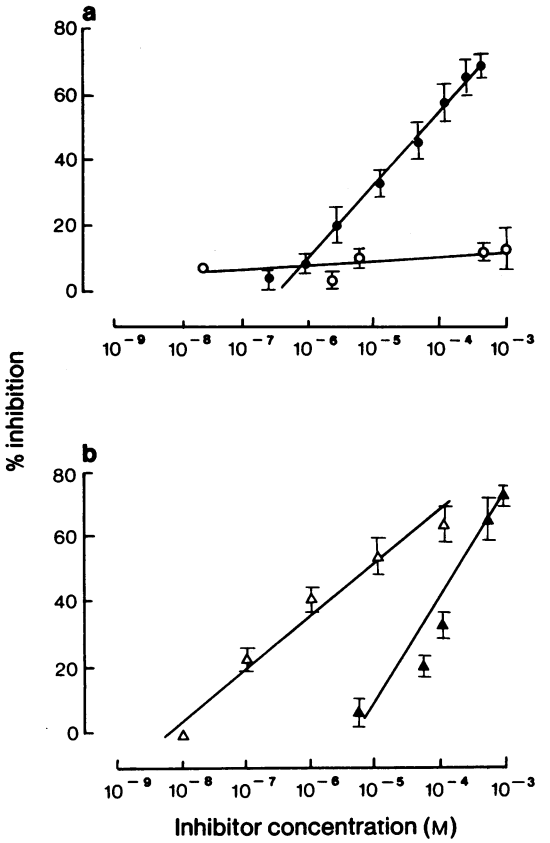


**Figure 1** Inhibition in rabbit colon of prostaglandin synthesis (open symbols) and prostaglandin metabolism (closed symbols) by sulphasalazine (a) and indomethacin (b). Synthesis experiments: resuspended colon microsomes incubated 60 min at 37°C with arachidonic acid (10 μg/ml) and cofactors. Metabolism experiments: 15 min incubations at 37°C with radiolabelled $PGF_{2\alpha}$ (10 μg/ml). Amount of prostaglandin synthesized or metabolized in absence of drug taken as 100%. Vertical lines indicate s.e. means, and points represent 3 to 6 determinations at each concentration.

tissue). However, in an experiment with rabbit lung 100,000 *g* supernatants and radiolabelled 15-keto $PGF_{2\alpha}$ as substrate, sulphasalazine did not inhibit prostaglandin Δ-13 reductase over the concentration range 0.25 to 500 μM. The principal metabolites of sulphasalazine formed by bacterial action in the gut are sulphapyridine and 5-aminosalicylic acid (Goldman & Peppercorn, 1975). Neither of these compounds significantly inhibited $PGF_{2\alpha}$ metabolism by rabbit colon supernatants at concentrations up to 5 mM (4 experiments).

**Discussion** These experiments show that sulphasalazine is a potent inhibitor of the initial step in $PGF_{2\alpha}$ metabolism, catalysed by 15-PGDH, but not of the second step (prostaglandin Δ-13 reductase) or of prostaglandin synthesis. This property is not shared by the two major metabolites of sulphasalazine. It is therefore possible that after sulphasalazine administration the actions of prostaglandins *in vivo* might be potentiated as a result of reduced inactivation. In preliminary experiments (Moore, Hoult & Laurie, 1978) we have found that sulphasalazine fed to rats in the diet at doses comparable to those used therapeutically in man attains concentrations sufficient to inhibit rat colon 15-PGDH *in vitro* (by 38.7 to 54.8%) but does not inhibit 15-PGDH in kidney or lung of the same animals. Taken together, this evidence suggests that inhibition of 15-PGDH by sulphasalazine in the colon, and consequent reversal of any state of relative prostaglandin deficiency which may prevail, may form the basis for the therapeutic benefit of the drug for the prevention of relapse of ulcerative colitis, since it is believed that prostaglandin deficiency may contribute to the pathogenesis of certain ulcers (Hinsdale, Engel & Wilson, 1974; Robert, 1976). It is also known that aspirin-like drugs (inhibitors of prostaglandin synthesis, see Flower, 1974) cause gastric and duodenal ulcers in experimental animals (Lee, Mollison & Cheng, 1971; Robert, 1976) and also in man (Rothermich, 1966) and that certain prostaglandins (e.g., $PGE_2$ and its analogues) may be used to aid the healing of stomach ulcers (Robert, 1976; Karim & Fung, 1976) and to protect against the ulcerogenic actions of aspirin-like drugs (Lippmann, 1974; Robert, 1976). Finally, carbenoxolone, a drug of therapeutic value in the treatment of gastric and duodenal ulcer, has been shown to be a potent inhibitor of 15-PGDH prepared from human gastric mucosa (Peskar, Holland & Peskar, 1976).

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