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EFFECTS OF SULPHASALAZINE AND ITS METABOLITES ON PROSTAGLANDIN SYNTHESIS, INACTIVATION AND ACTIONS ON SMOOTH MUSCLE

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1 We have investigated the effects of sulphasalazine and of its principal colonic metabolites (5-aminosalicylic acid and sulphapyridine) on prostaglandin inactivation, synthesis and actions on gastrointestinal smooth muscle.

2 Sulphasalazine inhibits prostaglandin $F_{2\alpha}$ breakdown in 100,000 *g* supernatants in all organs so far tested from 7 species with an ID_{50} of approx. 50 μM; it has a selective action on prostaglandin 15-hydroxydehydrogenase and does not inhibit prostaglandin Δ-13 reductase, prostaglandin 9-hydroxydehydrogenase or 'enzyme X' at millimolar concentrations. Enzyme activities were measured radiochemically or by bioassay.

3 Sulphapyridine and 5-aminosalicylic acid do not inhibit prostaglandin inactivation *in vitro* (4 species tested). A methyl analogue of sulphasalazine is a more potent inhibitor than the parent compound. Rabbit colon prostaglandin $F_{2\alpha}$ metabolism *in vitro* was inhibited by the following drugs with ID_{50} values (μM) of: diphloretin phosphate 20, sulphasalazine 50, indomethacin 220, frusemide 1000 and aspirin 10,000. A similar rank order of potencies was obtained with rabbit kidney.

4 Sulphasalazine at 50 to 100 μM inhibited inactivation of prostaglandin E_2 in the perfused rat and guinea-pig lung by 3 to 40% (rat) and 32 to 100% (guinea-pig) when measured by superfusion cascade bioassay and of prostaglandin $F_{2\alpha}$ by 43.6 ± 6.5% in rat lung perfused with 50 μM sulphasalazine and assayed radiochemically.

5 Prostaglandins E_1 and E_2 were 97.0 ± 8.2% and 92.3 ± 6.8% inactivated in the lungs after intravenous injection in the anaesthetized rat as measured by reference to their vasodepressor potencies when injected intra-arterially. Prostaglandin A_2 was not similarly inactivated. Pulmonary inactivation was prevented in the presence of an intravenous infusion of 16.3 μg kg^{-1} min^{-1} sulphasalazine and partially inhibited at a lower infusion rate.

6 Prostaglandin biosynthesis from arachidonic acid was measured in microsomal preparations from four sources by bioassay and radiochemical methods. Indomethacin was a potent inhibitor (ID_{50} 0.8 to 4.1 μM) but sulphasalazine and its methyl analogue were very weak inhibitors (ID_{50} 1500 to > 5000 μM), 5-aminosalicylic acid was weaker still and sulphapyridine inactive.

7 Sulphasalazine at 50 μM did not affect the actions of prostaglandins on five smooth muscle preparations; at 500 μM there was a rapidly reversible and probably non-specific antagonism of responses to low doses of prostaglandins.

8 The specificity and selectivity of the interaction of sulphasalazine and its metabolites with the formation, breakdown and actions of prostaglandins are discussed.

Introduction

We have shown recently that sulphasalazine is a potent inhibitor of prostaglandin inactivation in cell-free homogenates but is only a feeble inhibitor of prostaglandin biosynthesis from arachidonic acid (Hoult & Moore, 1978). In a separate study it was found that concentrations of sulphasalazine sufficient to inhibit prostaglandin breakdown were achieved in colon (but not in lung or kidney) after treating rats chronically with oral doses comparable to those used clinically in the treatment of ulcerative colitis (Moore, Hoult & Laurie, 1978). In view of these findings it was suggested that the beneficial therapeutic effects of this

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drug for the long term prevention of relapse in ulcerative colitis might be due to potentiation of prostaglandin actions in the colon consequent to reduced breakdown. It is already well established that prostaglandins have cytoprotective anti-ulcer actions in several species including man (reviewed by Karim & Fung, 1976; Robert, 1976; 1977).

The objectives of this study were to investigate the action of sulphasalazine on prostaglandin breakdown in more detail by measuring its effect *in vitro* on several enzymes important in prostaglandin metabolism and to determine its capacity to inhibit metabolism in intact cells in the whole organ. For this purpose we have studied pulmonary prostaglandin inactivation in the isolated perfused lung and in the anaesthetized rat. In addition we wished to examine the effect of sulphasalazine as well as of its metabolites sulphapyridine and 5-aminosalicylic acid on prostaglandin synthesis and on the spasmogenic actions of prostaglandins on smooth muscle. Some of these results have been communicated to the Society in preliminary form (Hoult, Moore & Ramcharan, 1979).

Methods

Prostaglandin inactivation in cell-free supernatants (see Hoult & Moore, 1977)

Tissues were homogenized in 50 mM phosphate buffer, pH 7.4, containing 1 mM disodium edetate (EDTA) and cysteine, and 100,000 *g* supernatants prepared. For radiochemical experiments, 10 μg/ml prostaglandin $F_{2\alpha}$ (or metabolite) labelled with 0.04 to 0.15 μCi tritiated prostaglandin (see below) was incubated at 37°C with the appropriate cofactor (5 mM) and supernatant, and extracted at timed intervals for thin layer chromatography. After chromatography on plastic-backed, silica gel-coated sheets in a solvent containing ethyl acetate: acetone: glacial acetic acid (90:10:1 v/v), breakdown of prostaglandin was assessed by comparing the proportion of counts in different zones of the chromatogram after localization of the position of metabolites by parallel chromatography of reference standards. Some sulphasalazine was also extracted but did not affect the migration of prostaglandins.

Prostaglandin breakdown was also measured by biological assay as previously described, either by direct transfer from the incubations or after extraction and reconstitution in Krebs solution. Samples were assayed against authentic prostaglandin standards on the rat isolated stomach fundus strip preparation bathed in Krebs solution containing a 'mixture' of antagonists.

Prostaglandin $F_{2\alpha}$ was the usual substrate, except for assay of prostaglandin Δ-13 reductase (15-keto prostaglandin $F_{2\alpha}$) and prostaglandin 9-hydroxydehydrogenase and 'enzyme X' (13, 14 dihydro-15-keto prostaglandin $F_{2\alpha}$).

Prostaglandin inactivation in perfused lungs

After decapitation, lungs were rapidly removed and perfused at 6 ml/min with warmed and well-oxygenated Krebs solution via a cannula inserted into the pulmonary artery. The perfusate was either passed over a superfusion cascade bioassay or collected for extraction of prostaglandins and radiochromatography.

The cascade usually consisted of two rat fundus strips and a rat colon superfused with a Krebs solution containing antagonists (Berry, Hoult, Littleton, Moore & Umney, 1979) and pulmonary prostaglandin inactivation was estimated by comparing responses to prostaglandins injected directly over the tissues or through the lungs. For radiochemical experiments, the lungs were perfused with 100 ng prostaglandin $F_{2\alpha}$ labelled with 0.07 μCi [9β-^{3}H]-prostaglandin $F_{2\alpha}$ and effluent collected for 3 min. After concentration of the aqueous phase, prostaglandins were extracted, redissolved in a small aliquot of methanol and subjected to chromatographic analysis and estimation of prostaglandin metabolites as described above.

For both types of experiment lungs were first exposed to prostaglandins when perfused with normal Krebs solution; this was then replaced with one containing 50 or 100 μM sulphasalazine.

Pulmonary prostaglandin inactivation in vivo

Male Sprague-Dawley rats (150 to 250 g) were anaesthetized with urethane (650 mg/kg i.p. followed after 5 min by the same dose s.c.) and polyethylene cannulae inserted into a femoral vein and the right carotid artery for intravenous and intra-arterial injections respectively. Blood pressure was recorded from a femoral artery. Sulphasalazine was administered by continuous slow intravenous infusion (1 ml every 30 min) at doses of up to 16.3 μg kg^{-1} min^{-1}. Pulmonary inactivation of prostaglandins was estimated by comparison of their vasodepressor effects when given via intravenous and intra-arterial routes (Horton & Jones, 1969).

Prostaglandin biosynthesis from arachidonic acid

Microsomal pellets from freshly centrifuged (100,000 *g*) homogenates were resuspended in pH 7.4 phosphate buffer containing EDTA and cysteine (both 1 mM). The following organs were studied: rabbit stomach, rabbit colon, guinea-pig kidney, sheep seminal vesicle, human placenta.

In all cases, arachidonic acid 10 μg/ml and reduced glutathione 3.0 mM were added and the samples incubated for 60 min and extracted into ethyl acetate after acidification. For radiochemical experiments, the incubations also contained 0.04 μCi [1-^{14}C]-arachidonic acid. After removal of the organic phase, samples were reconstituted in Krebs solution for bioassay on the rat isolated stomach strip preparation (bathed in a Krebs solution containing antagonists and using prostaglandins E_2 or $F_{2\alpha}$ as reference standard), or taken up in methanol and subjected to thin layer chromatography for 90 min in ethyl acetate:acetic acid:2,2,4-trimethylpentane:water (90:20:50:100, v/v). Chromatography in this solvent at room temperature afforded separation of the principal prostanoate products of arachidonic acid metabolism according to the migration of authentic reference standards, and the following approximate R_F values were obtained: prostaglandin $F_{2\alpha}$ 0.41, prostaglandin E_2 0.57, thromboxane B_2 0.45, 6-keto prostaglandin $F_{1\alpha}$ 0.23, and arachidonic acid 0.92. The percentage conversion to different products was estimated by sectioning the whole chromatogram and comparing radioactivity in each zone after suitable corrections for blank values obtained from samples extracted without incubation.

Actions of prostaglandins on isolated gastrointestinal smooth muscle preparations

The following preparations were studied: rabbit duodenum, guinea-pig ileum, guinea-pig oesophageal spiral, rat colon and rat stomach fundus strip. They were mounted in 3 ml organ baths and suspended in well-oxygenated Krebs solution at 37°C. Contractions were recorded isotonically with frontal writing levers or, in the case of the rat stomach strip and guinea-pig oesophagus, with an isometric strain gauge coupled to a pen recorder (Grass FT10 and polygraph). Dose-response curves to prostaglandins E_2 or $F_{2\alpha}$ were established under four successive conditons: normal Krebs, Krebs + 50 μM sulphasalazine, Krebs + 500 μM sulphasalazine, normal Krebs.

Animals and chemicals

The following animals were obtained from accredited suppliers: male Sprague-Dawley rats (120 to 300 g), male golden hamsters (80 to 200 g), male Dutch rabbits (1.5 to 3.0 kg), male Duncan-Hartley guinea-pigs (400 to 800 g) male and female cats (2 to 4 kg), male and female White Cross chicks (50 to 125 g). Human placentae were obtained at full term from normal deliveries and stored frozen.

Radiolabelled compounds were purchased from the Radiochemical Centre, Amersham: [1-^{14}C]-arachidonic acid (specific activity 58 mCi/mmol), [9β-^{3}H]-prostaglandin $F_{2\alpha}$ (sp. act. 14.4 Ci/mmol and also kindly supplied by Dr A. Brash at sp. act. 81.6 mCi/mmol) and [6-^{3}H]-13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (sp. act. 75 Ci/mmol). [9β-^{3}H]-15-keto prostaglandin $F_{2\alpha}$ (sp. act. 81.6 mCi/mmol) was synthesized biologically using rabbit colon 100,000 *g* supernatant and purified by thin layer chromatography as described previously (Moore & Hoult, 1978b).

We are grateful for the following gifts of chemicals: sulphasalazine and its methyl analogue (Pharmacia AB, Sweden), 5-aminosalicylic acid and sulphapyridine (May and Baker), diphloretin phosphate (Leo AB, Sweden), and frusemide (Hoechst).

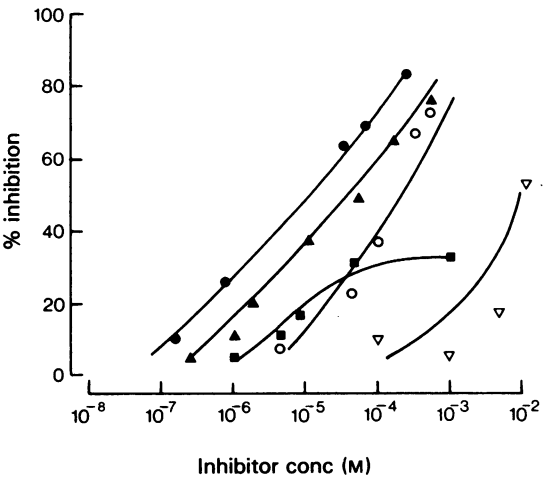

Figure 1 Inhibition by various drugs of prostaglandin $F_{2\alpha}$ breakdown in rabbit colon 100,000 *g* supernatants: 10 μg/ml radiolabelled prostaglandin $F_{2\alpha}$ was incubated with supernatants containing 5 mM NAD^+ for 15 min at 37°C and metabolism measured by radio-thin layer chromatography. Breakdown in absence of drug was set at 100%. (●) diphloretin phosphate; (▲) sulphasalazine; (○) indomethacin; (■) frusemide; (▽) aspirin. Points show means of 3 to 6 replicates at each concentration.

Results

Effect of sulphasalazine on prostaglandin breakdown in cell-free homogenates

The inactivation of 10 μg/ml (28.2 μM) prostaglandin $F_{2\alpha}$ was measured in high-speed 100,000 *g* cytoplasmic supernatants. In the presence of sulphasalazine there was a dose-related inhibition of metabolism as determined both by biological assay and by radiochromatography. Figure 1 shows that the ID_{50} of sulphasalazine in rabbit colon supernatant was 50 μM.

Several other compounds, including the aspirin-like non-steroidal anti-inflammatory drugs (see Flower, 1974; Hansen, 1976), diphloretin phosphate (Crutchley & Piper, 1974) and certain diuretics (see Hansen, 1976), are also known to inhibit prostaglandin breakdown. The effects of some of these drugs on prostaglandin $F_{2\alpha}$ inactivation were tested on the rabbit colon preparation (Figure 1). Approximate ID_{50} values are: diphloretin phosphate 20 μM, indomethacin 220 μM, frusemide 1000 μM and aspirin 10,000 μM. A similar rank order of inhibitory potencies of these drugs was obtained in experiments on rabbit kidney: diphloretin phosphate 28 μM, sulphasalazine 64 μM, indomethacin 340 μM and aspirin 1100 μM.

We have found that this inhibitory action of sulphasalazine on prostaglandin breakdown *in vitro* occurs in all organs and in all species so far tested (Table 1) and that 50 μM sulphasalazine produces inhibition ranging from 43.9% to 72.0%, with a mean value of 54.3 ± 2.0% in the 15 different supernatants studied. Seven species were tested, using organs which contain large amounts of prostaglandin metabolizing enzymes (e.g. lung, kidney and gastrointestinal tract).

The methyl analogue of sulphasalazine (i.e. 5-[4-(3-methyl-2-pyridylsulphamoyl)phenylazo]-2-hydroxybenzoic acid) was also tested and found to be more potent than the parent molecule as an inhibitor of prostaglandin breakdown in three of the four systems tested (Table 1).

Since the enzyme responsible for the initial step in the inactivation of classical prostaglandins is prostaglandin 15-hydroxydehydrogenase (Änggård, 1971; Samuelsson, 1972), we attribute the results described above to inhibition of this enzyme. We have tested the effects of sulphasalazine on three other enzymes concerned with prostaglandin metabolism: prostaglandin Δ-13 reductase, prostaglandin 9-hydroxydehydrogenase and 'enzyme X'.

Prostaglandin Δ-13 reductase converts 15-keto prostaglandins to the even less active 13,14-dihydro-15-keto metabolites (Änggård & Larsson, 1971; Lee & Levine, 1974; Westbrook & Jarabak, 1975), and its activity in rabbit lung supernatants was measured radiochemically by incubation with labelled 15-keto prostaglandin $F_{2\alpha}$. No inhibition by sulphasalazine was demonstrable even at 5 mM.

Prostaglandin 9-hydroxydehydrogenase oxidizes the 9-hydroxyl of F-series prostaglandins to a ketone group, thereby forming the corresponding E-series prostaglandin (Pace-Asciak & Miller, 1974). In rat kidney this conversion is effected at the level of the 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ metabolite (Pace-Asciak, 1975; Hoult & Moore, 1977), and the reaction was measured radiochemically using the appropriate substrate. No inhibition was observed with sulphasalazine at any of four concentrations up to 5 mM (e.g. Figure 2a). In rabbit kidney, 9-hydroxydehydrogenase is also capable of the direct conversion

Table 1 Inhibition by 50 μM sulphasalazine or its methyl analogue of prostaglandin $F_{2\alpha}$ inactivation in 100,000 *g* supernatants

Source of supernatant	*Inhibition (%)** *Sulphasalazine*	*Methyl analogue*
Rabbit colon	50.0 ± 4.1	80.0 ± 1.9
Rabbit stomach	61.0 ± 5.2	82.2 ± 4.1
Rabbit kidney	64.2 ± 5.3	
Rabbit lung†	48.2 ± 1.9	
Cat lung	58.2 ± 7.1	
Cat stomach	44.9 ± 2.1	
Human placenta	72.0 ± 5.1	72.7 ± 3.1
Chick kidney†	46.2 ± 1.9	
Chick lung†	58.2 ± 4.6	
Rat colon†	43.9 ± 6.5	
Rat kidney†	53.3 ± 7.5	
Rat lung†	53.6 ± 5.7	72.1 ± 1.4
Guinea-pig kidney	52.4 ± 5.1	
Guinea-pig lung	62.0 ± 9.7	
Hamster kidney	46.4 ± 14.7	
Mean value	54.3 ± 2.0	

* Expressed relative to control incubation lacking drug; radiochemical experiments were performed as described under Methods using 10 μg/ml prostaglandin $F_{2\alpha}$ and incubation times selected so that 40 to 80% breakdown occurred in control tubes. Results show mean ± s.e.mean for 4 to 6 determinations.
† Values taken from Hoult & Moore (1978).

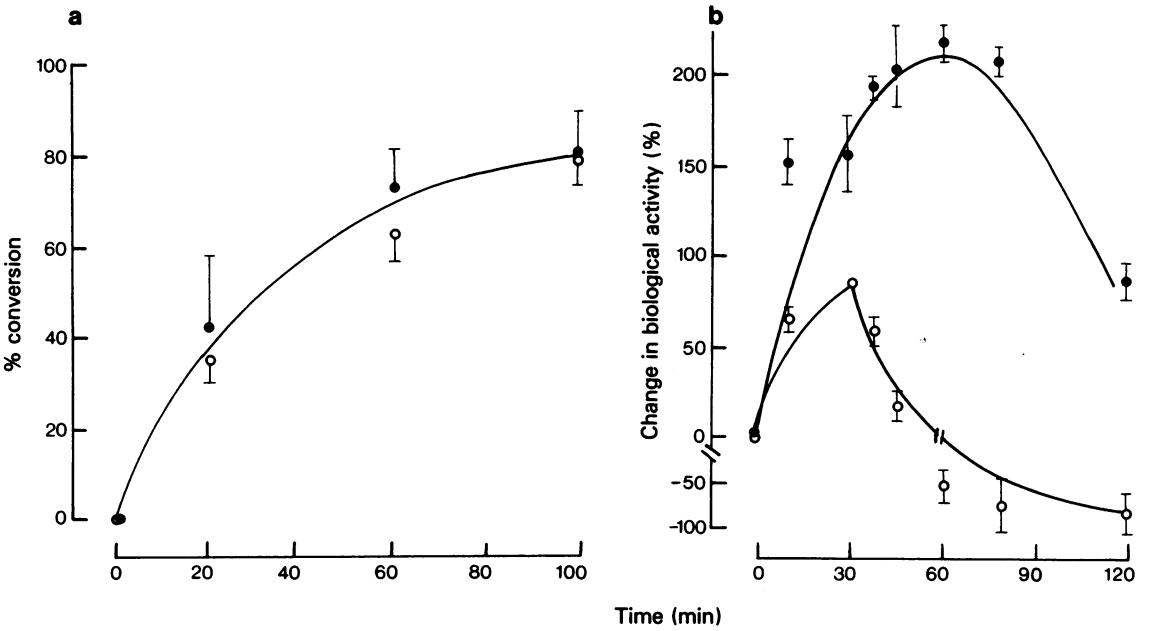

Figure 2 Effect of sulphasalazine on prostaglandin 9-hydroxydehydrogenase activity in renal homogenates of rat (a) and rabbit (b). (a) Rat kidney 100,000 *g* supernatant incubated with 0.1 μCi tritium-labelled 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (10 μg/ml) in the presence (●) and absence (○) of 5 mM sulphasalazine. Conversion to E series prostaglandin was assayed radiochemically. (b) Rabbit kidney 100,000 *g* supernatant incubated with 10 μg/ml prostaglandin $F_{2\alpha}$ and 5 mM NAD^+ in presence (●) and absence (○) of 100 μM sulphasalazine. Biological activity was assayed on the rat fundus strip in terms of prostaglandin $F_{2\alpha}$ equivalents and the changes in activity in the incubations expressed in terms of the values at time zero. In both graphs, results are the means of 4 determinations and vertical bars show s.e. mean.

of prostaglandin $F_{2\alpha}$ to prostaglandin E_2 (Hoult & Moore, 1977; Moore & Hoult, 1978a) and this was assayed biologically on the rat stomach strip preparation (Figure 2b). In the presence of 100 μM sulphasalazine a larger increase in biological activity was observed, suggesting enhanced formation of prostaglandin E_2. These results imply that although in this organ 15-hydroxydehydrogenase was inhibited by sulphasalazine (thus permitting a larger accumulation of prostaglandin E_2 before it is itself broken down), there was no inhibition of 9-hydroxydehydrogenase.

We have previously suggested the provisional name 'enzyme X' for the enzyme which converts 13,14-dihydro-15-keto prostaglandins to their 13,14-dihydro counterparts (Moore & Hoult, 1978b). It is found in rabbit liver (Moore & Hoult, 1978b), pig and guinea-pig kidney (Hoult & Moore, 1977) and in guinea-pig liver (Hamberg, Israelsson & Samuelsson, 1971). The name 'prostaglandin 15-keto reductase' was used by Änggård (1971) to describe this enzymatic transformation in homogenates of various organs of the swine, but detailed characterization and identification of a distinct enzyme has not been undertaken.

An experiment using bioassay to measure 'enzyme X' activity in guinea-pig liver homogenate showed that it was not inhibited by 100 μM sulphasalazine (Figure 3).

Effect of sulphapyridine and 5-aminosalicylic acid on prostaglandin inactivation

Sulphapyridine and 5-aminosalicylic acid are formed from sulphasalazine by bacterial action in the colon of man and other animals, and it has been suggested that one or both of these substances may be the therapeutically active constituent of sulphasalazine (Goldman & Peppercorn, 1975; Eastwood & Das, 1975). Neither of these drugs inhibited prostaglandin inactivation in high-speed supernatants at concentrations in the millimolar range when tested on homogenates from a variety of sources (Table 2).

Effect of sulphasalazine and its metabolites on prostaglandin synthesis

Prostaglandin synthesis from arachidonic acid was measured in four different microsomal 'prostaglandin

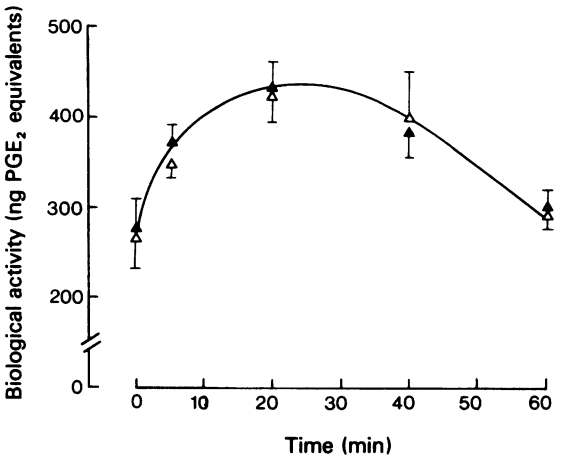

Figure 3 Effect of 50 μM sulphasalazine on 'enzyme X' activity in guinea-pig liver 14,000 *g* supernatants incubated with 10 μg/ml 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ and 5 mM NAD^+. The incubations were assayed in terms of prostaglandin E_2 equivalents on the rat fundus strip preparation: (▲) sulphasalazine; (△) control. Conversion of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ to 13,14-dehydro prostaglandin $F_{2\alpha}$ leads to an increase in smooth muscle activity (relative potency on this preparation of the two metabolites is 1:3), and subsequent transformation of the prostaglandin is to less active metabolites. Results show means of 4 replicates and vertical bars are s.e. mean.

synthetase' preparations (rabbit stomach, rabbit colon, guinea-pig kidney and sheep seminal vesicle) by bioassay and radiochemical methods. The actions of sulphasalazine, its methyl analogue and the colonic metabolites sulphapyridine and 5-aminosalicylic acid were compared with those of the potent prostaglandin synthetase inhibitor, indomethacin (Table 3). Indomethacin produced dose-related inhibition with approximate ID_{50}s in the range 0.75 to 4.1 μM, depending on the source of the microsomes; sulphasalazine and its methyl analogue were weak inhibitors with approximate ID_{50} values ranging from 1500 μM to more than 5000 μM; 5-aminosalicylic acid produced very little inhibition except at 1 mM in two systems and sulphapyridine was inactive. The results shown in Table 3 were obtained by measuring prostaglandin synthesis radiochemically; similar results for the inhibitory profiles of these drugs were obtained by bioassay of the samples obtained in experiments on rabbit colon and stomach microsomes. Radiochemical experiments on microsomes from human placenta gave similar results, with approximate ID_{50} concentrations against prostaglandin synthesis of: indomethacin <5 μM, sulphasalazine and methyl sulphasalazine ~1 mM, 5-aminosalicylic acid ≫1 mM, sulphapyridine inactive.

Effect of sulphasalazine on prostaglandin metabolism in the isolated perfused lung

There is extensive inactivation of the classical prostaglandins in the isolated perfused lung. We have measured such breakdown (a) in rat lungs after bolus injections of radiolabelled prostaglandin $F_{2\alpha}$ by extraction and radiochromatography, and (b) in both rat and guinea-pig lungs using superfusion cascade bioassay and prostaglandin E_2 as substrate. Addition of 50 to 100 μM sulphasalazine to the perfusing medium inhibited pulmonary prostaglandin inactivation in both species and according to both methods.

Figure 4 shows one example of several similar experiments using bioassay to estimate pulmonary inactivation of prostaglandin E_2; comparison of the doses required to elicit similar contractions of the assay tissue (in this case 14 mm contraction was selected arbitrarily) suggest that 93% of the prostaglandin is broken down after a single passage through the lungs, but in the presence of 100 μM sulphasalazine metabolism was reduced to 67%. Metabolism of prostaglandin E_2 was inhibited 3 to 40% in rat lung (7 experiments) and 32 to 100% in guinea-pig lung (3 experiments).

Table 2 Effect of sulphapyridine and 5-aminosalicylic acid on prostaglandin $F_{2\alpha}$ inactivation

	*Inhibition at 1 mM (%)**	
Source of 100,000 g supernatant	*5-Aminosalicylic acid*	*Sulphapyridine*
Rat stomach	0	0.2 ± 0.1
Rat kidney	0	0
Guinea-pig kidney	4.9 ± 2.2	0.7 ± 0.3
Rabbit colon	2.1 ± 1.8	0
Rabbit kidney	5.1 ± 3.9	0
Hamster lung	0	0

* Measured by radiochromatography as described in Table 1; results show mean ± s.e. mean for 4 determinations.

Two technical factors may limit the accuracy of these estimates of pulmonary prostaglandin inactivation and its reduction by pharmacological agents. Firstly, the tissue responsiveness to the prostaglandins appearing in the lung effluent may be altered by the presence of metabolites and second, the size and shape of the response may be affected by differences in the timing of exposure to prostaglandin resulting from the two routes of injection. Furthermore, in some experiments the dose-response curves for prostaglandin perfused through the lung in the presence of sulphasalazine were not parallel to the control curves, as illustrated in Figure 4. Sulphasalazine at these concentrations did not have any marked effect on the responsiveness of the tissues to prostaglandins injected directly over the tissues, at least at the two higher doses (Figure 4).

The separation of prostaglandin $F_{2\alpha}$ from its metabolites by thin layer radiochromatography affords a better quantitative estimate of the extent of inactivation in the perfused lung. The inactivation of a 100 ng bolus injection of prostaglandin $F_{2\alpha}$ ranged from 48.8 to 89.8% in lungs from four rats, and was reduced to 3.4 to 68.1% in the presence of 50 μM sulphasalazine (16 tests under both conditions). The combined results give a value of 43.6 ± 6.5% inhibition ($P < 0.01$, $n = 16$) at this drug concentration.

Effect of sulphasalazine on pulmonary prostaglandin inactivation in vivo

The pulmonary breakdown of prostaglandins in the anaesthetized rat was measured by comparison of the vasodepressor potencies of prostaglandins injected by the intravenous and intra-arterial routes (Horton & Jones, 1969; Crutchley & Piper, 1975). When injected intravenously prostaglandins E_1, E_2 and A_2 were vasodepressor in the rat with potencies relative to prostaglandin E_1 = 100% of 40% and 1.6% respectively; prostaglandin D_2 had variable effects and prostaglandin $F_{2\alpha}$ caused a rise in blood pressure. The intra-arterial dose-response curves to prostaglandin E_1 and E_2 lie far to the left of the intravenous curve (Figure 5a), showing that most of the injected dose is inactivated on passage through the lungs (prostaglandin $E_1 = 97.0 \pm 8.2\%$, prostaglandin $E_2 = 92.3 \pm 6.8\%$, $n = 5$). However, prostaglandin A_2 is not broken down and the two curves are superimposed (Figure 5b).

Concurrent slow intravenous infusion of sulphasalazine at 5.1 or 16.3 μg kg^{-1} min^{-1} caused a progressive shift of the intravenous dose-response curve of prostaglandin E_1 towards the intra-arterial curve, whereas the position of the latter remained unaltered (Figure 6). At the higher dose, substantial protection from pulmonary inactivation was obtained. These effects were not observed after single intravenous bolus injections of 10 to 50 μg of sulphasalazine.

In 3 experiments where survival of the animal permitted, it was found that recovery from sulphasalazine occurred within 20 to 40 min of stopping the infusion.

Effect of sulphasalazine on responsiveness of smooth muscle preparations to prostaglandins

The aspirin-like drugs have non-specific inhibitory actions on smooth muscle responsiveness to spasmogens (Northover, 1967; Manku & Horrobin, 1976; Famaey, Fontaine & Reuse, 1977), and diphloretin phosphate, although a potent inhibitor of prostaglandin breakdown, has also been shown to inhibit

Table 3 Dose-dependent inhibition of prostaglandin biosynthesis in various microsomal preparations by indomethacin, sulphasalazine and related drugs

		*Inhibition (%)**			
		Guinea-pig kidney	*Rabbit stomach*	*Rabbit colon*	*Sheep seminal vesicle*
Indomethacin	0.5 μM	10.9 ± 2.8	46.0 ± 2.2	22.0 ± 5.1	—
	5.0 μM	82.6 ± 5.1	71.0 ± 1.9	58.0 ± 6.1	—
Sulphasalazine	100 μM	0	21.0 ± 3.2	32.0 ± 2.2	3.9 ± 1.7
	1 mM	15.3 ± 4.9	46.0 ± 10.1	44.3 ± 5.1	35.8 ± 4.1
Methyl sulphasalazine	100 μM	0	0	12.3 ± 1.9	—
	1 mM	13.1 ± 4.1	44.0 ± 4.4	36.2 ± 2.1	—
5-Aminosalicylic acid	100 μM	0	17.0 ± 3.1	7.4 ± 4.1	1.2 ± 0.9
	1 mM	1.2 ± 0.9	25.0 ± 2.2	30.1 ± 2.9	0
Sulphapyridine	100 μM	0	4.2 ± 1.9	0	0
	1 mM	0	10.2 ± 5.0	0	11.2 ± 1.9

* Expressed relative to control incubation lacking drug; radiochemical experiments performed as described under Methods. Results show mean ± s.e. mean for 4 determinations at each concentration. – = not tested.

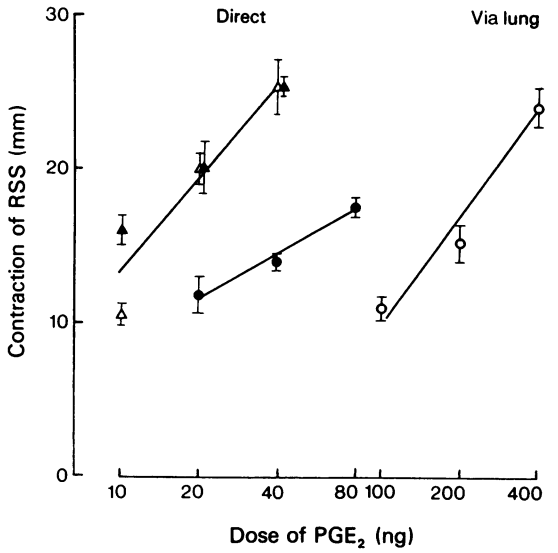

Figure 4 Inhibition of metabolism of prostaglandin E_2 by 100 μM sulphasalazine in the perfused rat lung. Ordinate scale shows contractions of rat stomach strip (RSS) to prostaglandin E_2 injected directly over the superfusion cascade (△, ▲) or via lung (○, ●). Open symbols: Krebs-perfused control; filled symbols: perfusion with Krebs + sulphasalazine. Points show means for 4 tests at each concentration and bars are s.e. mean.

some prostaglandin actions (Eakins, Fex, Fredholm, Högberg & Veige, 1973). The possibility that sulphasalazine might exert similar anti-prostaglandin effects was investigated using the rat stomach strip, rat colon, rabbit jejunum, guinea-pig oesophageal spiral and guinea-pig ileum preparations (3 experiments on each preparation). Figure 7 shows typical results for each of these tissues. Responses to prostaglandins were little changed in the presence of 50 μM sulphasalazine (in the 15 experiments, small potentiations were observed as often as small reductions or no change, see Figure 7), but were partially inhibited at 500 μM sulphasalazine, especially to the low doses of prostaglandin. Thus sulphasalazine is not an effective inhibitor of prostaglandin actions. We also found that the high concentrations of sulphasalazine inhibited the responses of these tissues to other agonists, although the depression was not as marked as to prostaglandins. The inhibitory effects of 500 μM sulphasalazine were reversed as soon as the bathing medium was replaced with normal Krebs solution. Taken together, the evidence summarized above suggests that high doses of sulphasalazine exert non-specific inhibitory effects on smooth muscle reactivity, perhaps by affecting ion fluxes or membrane permeability changes.

Discussion

These experiments provide evidence that sulphasalazine at micromolar concentrations inhibits prostaglandin inactivation in cell-free tissue homogenates prepared from a wide range of species and organs and that the drug has the same effect in the intact lung, both *in vitro* and in the anaesthetized rat. These *in vitro* properties are not shared by its principal colonic metabolites sulphapyridine and 5-aminosalicylic acid. The effects of these inactive metabolites on prostaglandin breakdown *in vivo* or in the perfused lung were not tested. Furthermore, the interrelationship of sulphasalazine with prostaglandins is specific: neither prostaglandin synthesis nor prostaglandin actions on gastrointestinal smooth muscle were inhibited except at very high doses. In addition, the metabolites were inactive against synthesis and in preliminary experiments appeared not to affect prostaglandin actions (Hoult & Moore, unpublished results).

It therefore seems reasonable to differentiate sulphasalazine from other drugs known to inhibit the formation, breakdown or actions of prostaglandins. Thus the aspirin-like non-steroidal anti-inflammatory drugs have an opposite spectrum of action since they are potent inhibitors of prostaglandin biosynthesis (Vane 1971; Flower, 1974), principally because of a direct action at the cyclo-oxygenase step (Roth, Stanford & Majerus, 1975; Vane, 1978), but have much less potent actions as inhibitors of metabolism (see Flower, 1974; Hansen, 1976; this study). Both sulphasalazine (see above) and the aspirin-like drugs are non-specific antagonists of prostaglandin responses (Northover, 1967; Manku & Horrobin, 1976; Famaey *et al.* 1977). Similarly, sulphasalazine is distinguishable from diphloretin phosphate, not only on the basis of structure but also because the latter drug is a relatively potent and specific prostaglandin antagonist, at least in some tissues (Eakins, Fex, Fredholm, Högberg & Veige, 1973). Although this is a useful property because specific prostaglandin receptor antagonists are not yet readily available, this action of diphloretin phosphate complicates its potential use in prostaglandin research as an inhibitor of breakdown. This limitation does not apply to sulphasalazine as there is a wide margin between doses which affect prostaglandin inactivation and actions, but as shown here it is less potent than diphloretin phosphate against prostaglandin inactivation *in vitro*.

The experiments using high-speed supernatants suggest that sulphasalazine inhibits prostaglandin breakdown by a specific action on prostaglandin 15-hydroxydehydrogenase because this is the first step in the catabolic pathway (references cited above). In addition sulphasalazine does not inhibit the actions of other prostaglandin metabolizing enzymes (for which we suggest the trivial name '*prostaglandinases*'), i.e.

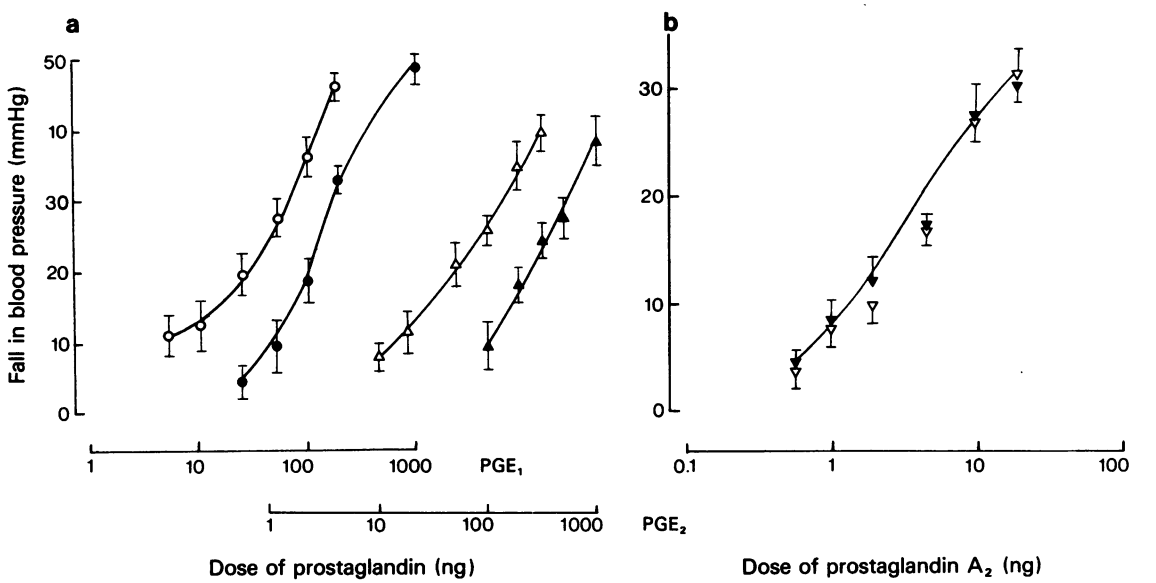

Figure 5 Effect of prostaglandins E_1 (○, ●), E_2 (△, ▲) in (a) and A_2 (▽, ▼) in (b) on the blood pressure of the anaesthetized rat. Vasodepressor responses (ordinate scale) were elicited by injections via the intra-arterial (open symbols) or intravenous routes (filled symbols), and the pulmonary inactivation of the prostaglandins estimated from the differences in these responses. Points show means for 4 to 17 tests at each concentration and bars are s.e. mean.

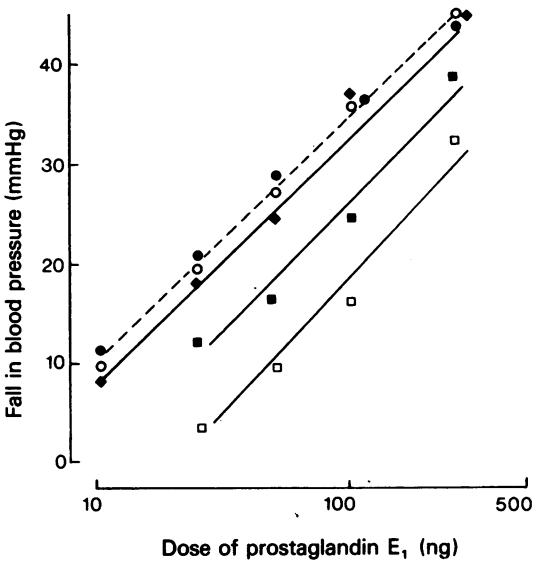

Figure 6 Effect of sulphasalazine on pulmonary inactivation of prostaglandin E_1 in the anaesthetized rat. Vasodepressor responses were measured in response to intravenous (□) and intra-arterial (○) injections of prostaglandin and pulmonary inactivation estimated from the difference in responses. Intravenous injections were also made during intravenous infusions of sulphasalazine at 5.1 μg kg^{-1} min^{-1} (■) and 16.3 μg kg^{-1} min^{-1} (◆); intra-arterial injections of prostaglandin E_1 were also made in the presence of sulphasalazine (●).

prostaglandin Δ-13 reductase, prostaglandin 9-hydroxydehydrogenase and 'enzyme X', even at millimolar concentrations. The nature of the inhibition, competitive or otherwise, has not been studied in the present experiments, although it appears from preliminary data that it is not due to competition with the obligatory NAD^+ cofactor for prostaglandin 15-hydroxydehydrogenase (Hoult & Moore, unpublished experiments). It would thus be of interest to study the effects of sulphasalazine on purified 15-hydroxydehydrogenase preparations as well as on other dehydrogenase enzymes. However, the powerful absorption spectrum of this compound in the range 320 to 390 nm precludes the use of the usual spectrophotometric methods for the measurement of NADH generated during such reactions. Similarly, the structure-activity relationships required for the inhibitory effect remain to be established, although we have shown above that the methyl analogue of sulphasalazine has at least equivalent potency.

Experiments with isolated perfused lungs of rat and guinea-pig and in the anaesthetized rat showed that sulphasalazine also inhibits prostaglandin breakdown in the intact lung. Since it is believed that catabolism in the lung is a two step process in which active transport of prostaglandin into the cell(s) containing prostaglandinases precedes enzymatic transformation (and is followed presumably by a third step, i.e. secretion or release of the metabolites), it is not possible to

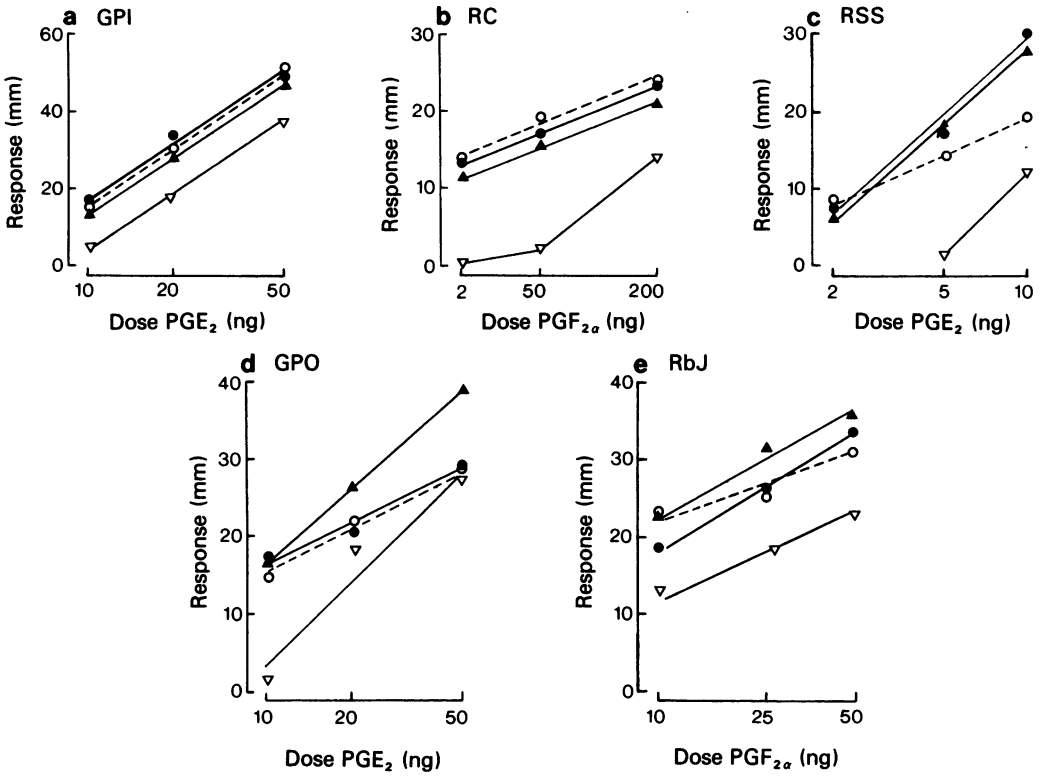

Figure 7 Effect of sulphasalazine on the responses of 5 isolated smooth muscle preparations to prostaglandin E_2 (PGE_2) or $PGF_{2\alpha}$: (a) guinea-pig ileum (GPI), (b) rat colon (RC), (c) rat stomach fundus strip (RSS), (d) guinea-pig oesophagus (GPO), (e) rabbit jejunum (RbJ). For experimental protocol, see Methods: (●) control tests in normal Krebs solution, (▲) in presence of 50 μM sulphasalazine, (▽) 500 μM sulphasalazine, (O----O) recovery in normal Krebs solution. Graphs show one typical experiment of three performed on each tissue and points represent the mean values for responses obtained with 3 to 8 tests at each dose. For clarity, the s.e. means have been omitted but in all cases were less than 15% of the mean and generally less than 8%.

specify the exact site of action of sulphasalazine in the lung. As yet there is little convincing direct evidence for prostaglandin transport (much being based on experiments with inhibitory dyestuffs of doubtful specificity) so it is not possible to measure any action of sulphasalazine on the transport step. However, in the light of the results presented above inhibition of intracellular 15-hydroxydehydrogenase seems more probable.

Complete inhibition of prostaglandin breakdown by the lung in the anaesthetized rat was observed at the higher infusion rate of sulphasalazine. As the infusion generally lasted for 20 to 30 min, allowing for a blood volume of 8 ml in a 200 g rat and discounting any breakdown of the drug, we calculate that the maximum plasma level was of the order of 30 μM. Furthermore, more than 90% of sulphasalazine in the blood stream is bound to plasma globulins (H. Schröder, personal communication). This suggests that *in vivo*, sulphasalazine is a more potent inhibitor of prostaglandin inactivation than the *in vitro* studies might indicate. It also appears to be considerably more potent *in vivo* than diphloretin phosphate: using rabbits, Crutchley & Piper (1975) obtained less complete inhibition of pulmonary prostaglandin E_2 inactivation with six fold larger infusions of diphloretin phosphate than those of sulphasalazine used here. Nevertheless, a large proportion of this drug is also thought to bind to plasma proteins.

These *in vivo* experiments confirmed previous observations concerning the vasodepressor potencies of prostaglandins E_1, E_2 and A_2 (see Nakano, 1973) and showed that the E-series compounds are extensively broken down on passage across the lungs (Vane, 1969; Gillis & Roth, 1976). Although it is known that prostaglandin A_2 is a reasonable substrate for 15-hydroxydehydrogenase (Nakano, Änggard & Samuelsson, 1969), it is not certain whether

the reason it escapes pulmonary inactivation *in vivo* is because it is not taken up by the transport mechanism (Anderson & Eling, 1976) or because it is bound to plasma proteins (Raz, 1972; Gueriguian, 1976).

We have suggested elsewhere that local inhibition of prostaglandin metabolism in the colon might explain the action of sulphasalazine when used prophylactically in the treatment of inflammatory bowel disease (Moore, Hoult & Laurie, 1978; Hoult & Moore, 1978). In contrast, other authors favour inhibition of prostaglandin synthesis by the parent drug or metabolite as a mechanism of action (Gould, 1975; Sharon, Ligumsky, Rachmilewitz & Zor, 1978), but this is hard to reconcile with the evidence presented above. Thus the ratios of potencies of anti-synthesis and anti-metabolism actions of sulphasalazine are opposite to those of the aspirin-like drugs and consequently aspirin-like anti-inflammatory properties would not be predicted for sulphasalazine. Indeed they have not been noted in clinical practice. Furthermore, potent prostaglandin synthesis inhibitors have not been reported to have beneficial effects in the treatment of ulcerative colitis despite suggestions that they be tested experimentally.

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