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Effects of aspirin, indomethacin, flufenamic acid and paracetamol on prostaglandin output from rat stomach and renal papilla in-vitro and ex-vivo

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The effects of aspirin, indomethacin, flufenamic acid and paracetamol on prostaglandin (PG) biosynthesis were studied in whole cell preparations of rat renal papilla and stomach in-vitro and ex-vivo. In the ex-vivo experiments a low dose aspirin was a potent inhibitor of PGE output from the stomach but not the renal papilla, while in-vitro renal PGE output was inhibited by aspirin to a greater extent than gastric PGE. Indomethacin and flufenamic acid inhibited both renal papillary and gastric PGE outputs in-vitro and ex-vivo. Paracetamol enhanced PGE output from the stomach more than twice ex-vivo, and to a lesser extent in-vitro. It also augmented PGE output from the papilla ex-vivo but not in-vitro. In view of the possible contribution of cellular organization and pharmacokinetic processes to the ultimate effect, it is suggested that studies on the effects of anti-inflammatory and antipyretic agents on PG biosynthesis should not be restricted to fully in-vitro systems.

Numerous investigations have shown gross agreement between the pharmacological potency of non-steroidal anti-inflammatory drugs (NSAID) and their in-vitro inhibition of prostaglandin (PG) synthesis (see Flower 1974). However, some notable exceptions have emerged, both in terms of anti-inflammatory activity (Deby et al 1975; Robak et al 1980) and adverse effects of the NSAID (Tachizawa et al 1977). Part of the apparent discrepancy may be attributed to differences in the properties of synthetase from different sources (Flower & Vane 1972; Bhattacherjee & Eakins 1974). Cellular organization and pharmacokinetic processes that may lead to differential distribution in tissues or subcellular compartments may also account for apparent selectivity on PG biosynthesis in different organs (Attallah & Stahl 1980; Brune et al 1976; Whittle et al 1980). In the present investigation the effects of several non-steroidal anti-inflammatory and antipyretic agents on PG output from whole cell preparations of two test organs have been compared. The rat stomach and renal papilla were chosen, as they represent major sites of adverse reactions to the NSAID. The drugs were either added to the incubation in-vitro, or administered to rats before their effect on PG output was assessed ex-vivo. We restricted our determinations to PGE_2, which is a predominant prostanoid elaborated in these tissues.

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MATERIALS AND METHODS

Female Charles-River rats, 170–220 g, from which food had been withdrawn for 24 h, while water was freely provided, were decapitated and the stomach and kidney immediately removed, rinsed and dissected. The gastric corpus (including mucosa and muscular layers) and renal papilla were cut into slices 1–2 mm each, and placed separately in Erlenmeyer flasks containing 2 ml each of Krebs-Henseleit bicarbonate buffer containing glucose (1 mg ml^{-1}). Tissues were incubated at 37 °C under 95% O_2 - 5% CO_2 and the buffer replaced every 30 min. The buffer samples that were recovered were frozen at the end of each experiment and were then directly assayed for PGE content by radioimmunoassay. Anti-PGE-BSA serum (Miles-Yeda Ltd, Rehovot, Israel) reacted equally with PGE_2 and PGA_2. PGE_1, PGA_1, PGB_2, and PGB_1 also cross-reacted with the antiserum, while $PGF_{2\alpha}$, PGD_2 and 6-Keto-$PGF_{1\alpha}$ did not produce significant cross reactions. Thus, the assay could identify prostaglandins of the E series, both monoenoic and dienoic, and their dehydration products, (PGA and PGB), which probably do not occur biologically, but are formed chemically during extraction (Moncada et al 1980). Also as rat tissues elaborate predominantly dienoic PGs, the results were expressed as PGE_2 equivalents. [^{3}H]PGE_2 (the Radiochemical Centre, Amersham) had a specific activity of 160 Ci $mmol^{-1}$. None of the drugs interfered with the assay at the concentrations used.

In the ex-vivo experiments drugs were injected subcutaneously in propylene glycol solutions, 2 h before decapitation of the rats. Control rats were injected with propylene glycol. In the in-vitro experiments the drugs were directly added with every change of the buffer.

Aspirin was obtained from BDH (Poole, UK), indomethacin and paracetamol from Sigma (St. Louis, Mo). Flufenamic acid was kindly donated by Rafa Labs, Jerusalem. All reagents were of analytical grade.

Statistical evaluation was effected by the rank sum test (Dixon & Massey 1969).

RESULTS

Control incubations displayed a pattern in which PGE output was higher initially and then gradually declined to a plateau after 60 to 90 min (not shown), as previously described for the rat renal papilla (Danon et al 1975) and for the stomach (Assouline et al 1977). PGE output from control renal papillary incubations at 90 min was 325 ± 41 ng/100 mg tissue/30 min, whereas that from gastric incubates was 4·4 ± 0·6 ng/100 mg tissue/30 min. Therefore the effects of the anti-inflammatory/antipyretic drugs on PGE output are shown in Fig. 1 relative to the controls at each incubation interval.

Ex-vivo experiments. In these trials the drugs were administered subcutaneously to rats, and the tissue slices then incubated without further drug additions. The doses of drugs were chosen, after preliminary trials, to produce appreciable but not complete inhibition of PGE output (or stimulation, as it turned out with paracetamol) in at least one of the test organs.

As shown in the left hand panels of Fig. 1, a relatively low dose of aspirin (10 mg kg^{-1}) produced significant inhibitions of PGE output from the stomach (78% initially) but was without effect on the renal papilla. On the other hand, indomethacin (20 mg kg^{-1}) inhibited PGE output from both test organs, producing 91% inhibition of initial PGE output in the papilla and 63% initial inhibition in the stomach. Flufenamic acid (100 mg kg^{-1}) produced an even greater initial inhibition of renal papillary than of gastric PGE output. Paracetamol (800 mg kg^{-1}) produced an unexpected greater than two-fold increase in PGE output from the stomach, as well as significant augmentation (at 60 and 90 min) of PGE output from the renal papilla.

Fig. 1 also indicates that while the effects of aspirin and flufenamic acid on PGE output were apparently

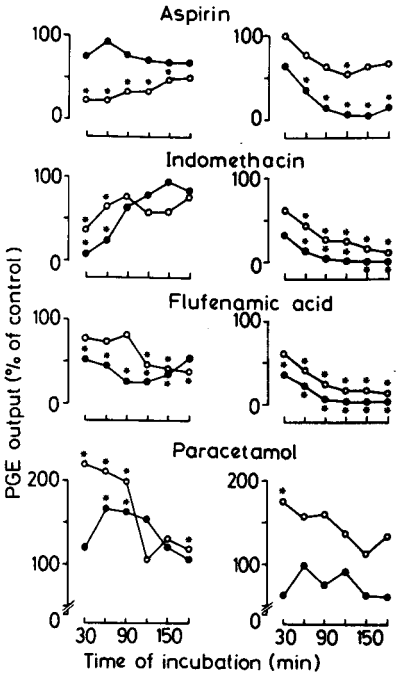

FIG. 1. Effects of anti-inflammatory/antipyretic drugs on PGE output ex-vivo (left hand panels) and in-vitro (right hand panels). Open circles represent PGE output from rat stomach, closed circles—renal papilla. PGE outputs, as effected by the different drugs used, are given as percent of controls (means of 5–7 experiments each). Asterisks denote statistically significant differences from controls ($P < 0.05$) by the rank sum test. The drug doses/concentrations used in the ex-vivo and in-vitro experiments, respectively, were: aspirin 10 mg kg^{-1}, 10 μg ml^{-1}; indomethacin 20 mg kg^{-1}, 1 μg ml^{-1}; flufenamic acid 100 mg kg^{-1}, 5 μg ml^{-1}; paracetamol 800 mg kg^{-1}, 50 μg ml^{-1}.

sustained throughout the incubation, those of indomethacin (inhibition) and of paracetamol (stimulation) were quickly washed out.

In-vitro experiments. Here the drugs were added in-vitro with every change of the incubation buffer. The drug concentrations were chosen after some preliminary trials (not shown). As depicted in the right hand panels of Fig. 1, the inhibitions showed a tendency to become more intense with time. The relative efficacy of aspirin in inhibiting PGE output from the two test organs in-vitro was reversed, compared with the ex-vivo trials. Thus, aspirin (10 μg ml^{-1}) produced up to 94% inhibition of PGE output from the papilla, while the same concentration had only a marginal effect on PGE output from the gastric tissue (significant only at 120 min). Indomethacin (1 μg ml^{-1}) and flufenamic acid

(5 μg ml^{-1}) produced large inhibitions of PGE output from both the renal papilla (99 and 96% maximal inhibitions respectively) and the stomach (87 and 83% inhibitions, respectively).

Paracetamol in-vitro (50 μg ml^{-1}) effected an increase in PGE output only from the stomach, reaching statistical significance at 30 min. However, the same dose failed to either significantly reduce or augment PGE output from the renal papilla.

DISCUSSION

The observation of selective effects of the non-steroidal anti-inflammatory/antipyretic drugs on the two test organs and their differential behaviour in-vitro and ex-vivo seems of particular interest. Thus, whereas aspirin was a much more potent inhibitor of PGE output in the renal papilla than in the stomach in-vitro, the reverse was found to occur ex-vivo. A dose which produced greater than 70% inhibition of gastric PGE output ex-vivo was without effect on the renal papilla. Selective inhibition of PG production in different tissues by anti-inflammatory compounds has been shown by others (Whittle et al 1980; Attallah & Stahl 1980). Obviously, the pharmacokinetic behaviour of aspirin may have contributed to the greater effect on the stomach than on the kidney in the ex-vivo experiments. It has been reported that salicylates as well as other acidic anti-inflammatory compounds may achieve much higher concentrations in the gastric mucosa than at other sites (Morris et al 1967; Brune et al 1977a, b; Garner 1978).

The stimulatory effect of paracetamol on PGE output from the stomach and renal papilla ex-vivo is intriguing. The effect of this antipyretic drug on PG biosynthesis has been disputed. Paracetamol was reported to be a potent inhibitor of PG synthetase in rabbit brain microsomes (Flower & Vane 1972), while in rat brain homogenates PG generation was hardly inhibited (Wolfe et al 1976). Robak et al (1978) described a biphasic effect of paracetamol on PG synthetase activity from bovine seminal vesicle microsomes in the absence of co-factors. At lower concentrations (67–667 μM), it stimulated PG generation, while higher concentrations were inhibitory. However, in the presence of glutathione and hydroquinone, paracetamol either had no effect or inhibited enzyme activity (IC50 = 1500 μM) (Robak et al 1978). Other authors had reported that paracetamol was inactive in inhibiting PG synthetase in dog spleen microsomes (Flower et al 1972) or in rat skin homogenates (Greaves & McDonald-Gibson 1972). Thus it appears that paracetamol may produce either inhibition, stimulation or no effect on PG synthesis in-vitro, depending on the enzyme source, drug concentration or experimental conditions. Few reports concerned themselves with the effect of paracetamol on PG biosynthesis in-vivo (Fitzpatrick & Wynalda 1976; Zenser et al 1978). The present results indicate that a high dose paracetamol greatly enhanced PGE generation by rat gastric as well as renal papillary slice preparations ex-vivo. Smaller doses had been tried in preliminary experiments (results not shown) and found to be stimulatory as well. The dose was subsequently increased to the highest tolerated dose (Mitchell et al 1973). Recently, paracetamol was reported not only to be devoid of any damaging effect on the gastric mucosa (Ivey et al 1978), but also to protect the rat gastric mucosa against aspirin-induced erosions (Seegers et al 1979). Thus, augmentation of PGE biosynthesis in the mucosa may provide the basis for the effect of paracetamol on aspirin-induced erosions. On the other hand, McDonald-Gibson & Collier (1979) suggested that paracetamol may render cyclo-oxygenase more vulnerable to inhibition by aspirin. Other drugs that enhance PG biosynthesis were recently developed and shown to be anti-inflammatory (Kuehl et al 1977; Robak et al 1980) and to protect the gastric mucosa against indomethacin-induced damage in rats (Pomarelli et al 1980).

The present data emphasize the need for more relevant information on the effects of anti-inflammatory/antipyretic agents on PG biosynthesis in-vivo. In spite of justifiable criticism relating to the possible interference of tissue metabolism with the overall amounts of PG recovered, ex-vivo experiments with whole-cell preparations may provide such information.

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