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The effect of an aqueous extract of comfrey on prostaglandin synthesis by rat isolated stomach

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Comfrey, a herb of wide and longstanding use as a home remedy for various complaints including rheumatism and gastric upsets, is usually administered as a tea (aqueous infusate of dried leaves). We therefore prepared an aqueous extract of comfrey, and because these complaints may involve prostaglandins we investigated the effect of the extract on prostaglandin synthesis. The rat stomach was chosen partly because we have studied its prostaglandin synthesis previously.

Methods

Dried comfrey leaves (*Symphytum officinale*) gathered as a mature crop on a herb farm in Germany, were homogenized in Krebs solution, filtered and a stock solution made equivalent to 10 mg dried leaves in 1 ml. The combined gastric corpus and antral mucosa and muscle from male Wistar rats (approximately 250 g, 8 rats/experiment) were cut into small pieces, mixed, and washed three times in Krebs solution. Weighed tissue samples (range 260–390 mg) were incubated for 30 min at 37 °C in 5 ml Krebs solution alone or containing different concentrations of comfrey extract (50 μg–5 mg ml^{-1}) to assist penetration to the prostaglandin synthetase. The tubes were put on ice for 15 min then removed and the contents were homogenized for 30 s using a Silverson homogenizer. A 1 ml aliquot was removed for further incubation with [1-^{14}C]arachidonic acid (0·08 μCi, 34 nM, 30 min 37 °C). All incubates were extracted for prostaglandins (Unger et al 1971). The unlabelled samples were bioassayed against PGE_2 on rat gastric fundus (Bennett et al 1973) as were extracted comfrey solutions (50 μg–5 mg ml^{-1}) incubated without stomach tissue. Following lipid extraction of the labelled arachidonic acid products, the samples were chromatographed on thin layer plates (organic phase of ethyl acetate–hexane–acetic acid–water, 56 : 24 : 12 : 60), and autoradiographs prepared (Kodak, NS-2T; exposure 10 days) (Cottee et al 1977).

In other experiments the gastric tissue (250–380 mg $5 ml^{-1}$; 1 rat/experiment) were incubated in Krebs solution alone (control) and in comfrey solution (5 mg ml^{-1}). Incubation, homogenization and extraction were performed as above and 20% of each extract was used for bioassay (Bennett et al 1973). The remainder was partly purified by C-18 reverse phase Sep-paks (Waters Associates) and, the prostaglandin fraction eluted with acetonitrile (modified from Powell 1980). Reverse phase h.p.l.c. was performed isocratically with acetonitrile–water–phosphoric acid, 30 : 70 : 0·1 at a flow rate of 1 ml min^{-1} (Van Rollins et al 1980). Standard curves for 6-keto-$PGF_{1\alpha}$ or $PGF_{2\alpha}$ or PGE_2 (0–250 ng) were linear.

Results

The values are presented as medians with semi-quartile ranges in parentheses, and analysed statistically using the Mann-Whitney U-test. The bioassay detected 100 pg PGE_2 ml^{-1}. Amounts of bioassayed prostaglandin-like material (PG-lm) extracted from samples incubated with comfrey extract equivalent to 50, 500 and 5000 μg comfrey ml^{-1} increased in a concentration-related manner (22–128% increase over controls; 3 experiments, duplicate assays, $P = 0·026$ to 0·002, Fig. 1). Lower concentrations (0·5 and 5 μg ml^{-1}) had no significant effect. Gastric tissues from individual rats yielded 35–60% more PG-lm with comfrey 5 mg ml^{-1}, compared with controls (n = 5, $P = 0·008$;

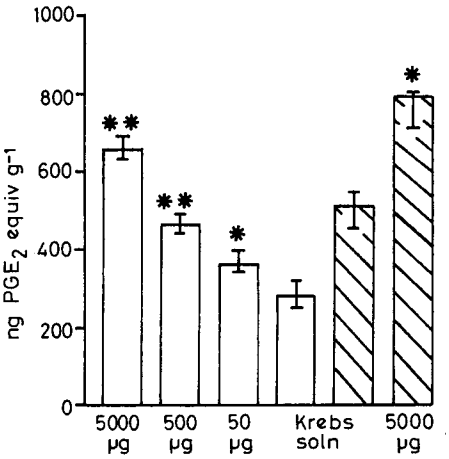

FIG. 1. Bioassayed material (assayed as ng PGE_2 equivalents g^{-1}) extracted from pooled tissues homogenized in Krebs solution alone and in Krebs solution containing different concentrations of comfrey (50–5000 μg ml^{-1}, duplicate assays in 3 experiments at each concentration). The columns are median values; the vertical bars represent semiquartile ranges. Hatched columns represent experiments using one rat per experiment (n = 5). *P* values, * < 0·05, ** < 0·01.

* Correspondence.

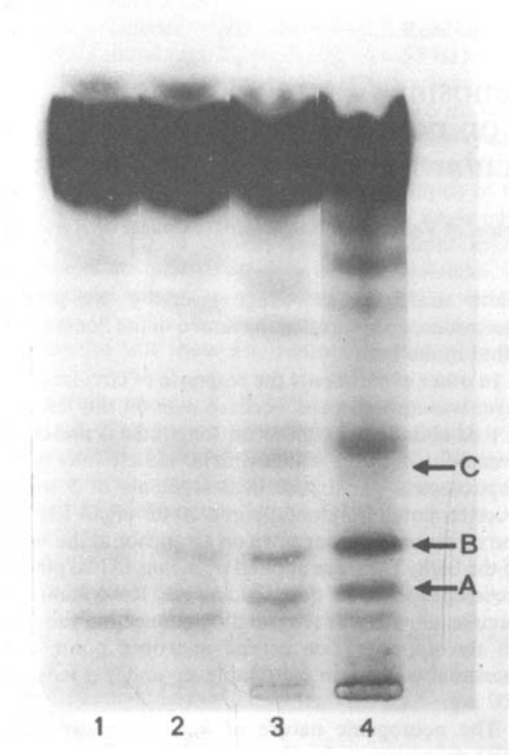

FIG. 2. An autoradiograph showing products of [1-^{14}C]-archidonate metabolism by rat stomach homogenates in 1, Krebs solution control; 2, 3 and 4, comfrey solution 50, 500 and 5000 μg ml^{-1}. Comfrey caused concentration-related increases in substances resembling 6-keto-$PGF_{1\alpha}$ (A), $PGF_{2\alpha}$ (B), and PGE_2 (C).

Fig. 1). Comfrey extracts alone processed similarly had little or no effect when added to the bioassay tissue, so that the assay results were due almost entirely to PG-1m released from incubated corpus and antrum.

Autoradiography demonstrated that comfrey caused a concentration-related increase in [1-^{14}C]arachidonate metabolism by rat stomach homogenates (Fig. 2). Using R_F characteristics, the greatest incorporation appears to be into $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, with less into PGE_2.

H.p.l.c. analysis shows that comfrey (5 mg ml^{-1}) increased the gastric output of $PGF_{2\alpha}$ by 40 to 360% ($n = 5$, $P < 0{\cdot}002$). 6-Keto-$PGF_{1\alpha}$ output also always increased (6–220%; $n = 5$, $P < 0{\cdot}1$), but the change in PGE_2 release was variable (−48 to 196%, $P > 0{\cdot}1$) (Fig. 3).

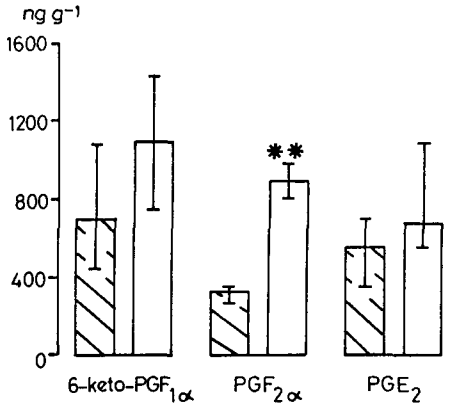

FIG. 3. H.p.l.c. analysis of compounds which co-eluted with 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2, expressed as ng g^{-1}. Hatched columns represent Krebs solution control extracts; open columns represent comfrey solution extracts 5 mg ml^{-1}. P value ** $< 0{\cdot}01$.

Discussion

The bioassay results demonstrate that an aqueous (Krebs solution) extract of comfrey (concentrations equivalent to 50 μg–5 mg dried leaf) increases the release of prostaglandin-like material from rat gastric corpus and antrum. Radioassay and h.p.l.c. indicated greater outputs of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. Since various prostaglandins can protect the gastric mucosa (Robert 1977) this might explain the use of comfrey leaves in gastric upsets. Comfrey leaves contain protein, pyrrolizidine alkaloids (Hirono et al 1978), allantoin, tannin and mucilage, but it is not known to what extent these contribute to the increased prostaglandin synthesis by rat stomach.

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