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Carbenoxolone and deglycyrrhized liquorice have little or no effect on prostanoid synthesis by rat gastric mucosa *ex vivo*

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1 Rats were given either carbenoxolone 50 mg kg^{-1}, deglycyrrhized liquorice 1 g kg^{-1} or vehicle by gastric tube. The doses were repeated 16 h later, and the stomachs removed after another 2 h.

2 The amounts of prostaglandin E (PGE), 6-keto-$PGF_{1\alpha}$ and thromboxane B_2, measured by radioimmunoassay in extracts of the gastric corpus and antrum mucosa, were similar in the treated animals and the controls.

3 We conclude that in rats, carbenoxolone and deglycyrrhized liquorice may exert their anti-ulcer effect by a non-prostaglandin mechanism. This contrasts with the mechanism thought to occur in man with carbenoxolone.

Introduction

The liquorice derivatives carbenoxolone and deglycyrrhized liquorice are used to treat peptic ulceration. Peskar *et al.* (1976) studied carbenoxolone for its effect on prostaglandin metabolism by human gastric mucosa *in vitro* because prostaglandins can protect the gastric mucosa from damage. They found that carbenoxolone 0.01–0.5 mM inhibited the mucosal 15-hydroxy-prostaglandin-dehydrogenase and prostaglandin-Δ^{13}-reductase. Further experiments by Peskar & Weiler (1983), using biopsies of human gastric mucosa, suggested that carbenoxolone may act not only by increasing the amount of prostaglandin E_2 (PGE_2) but also by reducing thromboxane B_2 (TXB_2) formation. *In vitro* experiments by Martin *et al.* (1983) demonstrated that carbenoxolone incubated with fragments of rat stomach increased the accumulation of PGE, but TXB_2 was not examined. The main purpose of the present experiments was therefore to determine *ex vivo*, in contrast to the *in vitro* studies above, whether carbenoxolone given to rats affects the amount of gastric mucosal prostanoids. Another aim was to compare the findings with deglycyrrhized liquorice (DGL) which is a related anti-ulcer preparation. A brief account of this work was presented at the 9th International Congress of Pharmacology (Melhuish *et al.*, 1984).

Methods

Male Sprague Dawley rats weighing 210–400 g were deprived of food at 10 h 00 min, but allowed free access to water. At 16 h 00 min they were weighed and given carbenoxolone 50 mg kg^{-1} or deglycyrrhized liquorice 1 g kg^{-1} (each made up freshly as an aqueous suspension in 1% gum arabic) by gastric intubation in a volume of 1.25 ml. These doses show anti-ulcer activity in rats (Khan & Sullivan, 1968; Rees *et al.*, 1979). The controls received vehicle alone. At 08 h 00 min the following day the dose was repeated and at 10 h 00 min the rats were killed by cervical dislocation.

The stomachs were removed quickly but gently into a Petri dish on ice, and opened along the greater curvature. Any residual gastric contents were removed by washing with cold Krebs solution. The mucosa from the corpus and antrum was removed from the muscle after injecting cold Krebs solution between

Table 1 Measurements of prostaglandin E (PGE), 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2) by radioimmunoassay of extracted rat corpus and antral mucosa

	Controls	*Carbenoxolone*	*DGL*
PGE	211(140–259)	239(225–310)	248(233–273)
6-KETO-$PGF_{1\alpha}$	320(241–554)	440(359–486)	401(306–245)
TXB_2	24(17–30)	25(19–28)	21(17–27)

The values are ng g^{-1} wet tissue, shown as medians with semiquartile ranges in parentheses. There were 28 rats in the control group (27 for TXB_2) and 14 rats in each of the other groups (13 for TXB_2/ carbenoxolone). DGL: deglycyrrhized liquorice.

them to facilitate separation using scissors. After weighing, the tissue was homogenized for 30 s (Silverson homogenizer) in Krebs solution: ethanol (50:50) acidified to about pH 3 with formic acid (Bennett *et al.*, 1973). This procedure yields the prostanoids present in the gastric mucosa, since the acid ethanol inhibits new formation during tissue processing. The prostanoids were extracted into chloroform (Unger *et al.*, 1971), the dried extract was mixed with tricene buffer, and the samples analysed quantitatively in duplicate by radioimmunoassay for PGE, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TXB_2). Percent cross reactions of the antibodies with other prostanoids are: PGE antibody, PGE_2 100, PGE_1 70, PGA_2 1, PGA_1 1, $PGF_{2\alpha}$ 5, $PGF_{1\alpha}$ 3, PGB_2 0.1, PGB_1 0.6; 6-keto-$PGF_{1\alpha}$ antibody, PGE_2 0.1, $PGF_{2\alpha}$ 3.0, TXB_2 0.02; TXB_2 antibody, PGE_2 <0.01, $PGF_{2\alpha}$ 0.11, 6-keto-$PGF_{1\alpha}$ 0.01%. The results are presented as median values with semiquartile ranges in parentheses, and analysed by the Mann-Whitney U-test.

In another experiment we showed that orally administered aspirin reduces the *ex vivo* yield of gastric mucosal prostaglandins in our rats.

Results

The yields of substances measured by radioimmunoassay for PGE, 6-keto-$PGF_{1\alpha}$ and TXB_2 in the carbenoxolone and DGL experiments are shown in Table 1. The drugs had little or no effect on the amounts of extracted prostanoids. All the comparisons are $P > 0.1$ except for PGE in the DGL group where the median value is 18% higher than in controls ($P = 0.1$).

Gastric mucosal formation of prostanoids could be affected by drugs, as shown by giving rats aspirin 60 mg ml^{-1} or vehicle by stomach tube. The animals were killed 2 h later, and the separated antral and corpus mucosae homogenized in acid ethanol. Amounts of PGE and 6-keto-$PGF_{1\alpha}$, measured by radioimmunoassay were: controls 178 (128–250) and 193 (139–228) ng g^{-1} wet weight respectively; aspirin-treated rats 28 (13–81) and 11 (7–20) ng g^{-1} respectively (19/group, both $P < 0.002$).

Discussion

The measurements of prostanoids approximate to their amounts in the mucosa, because new formation of prostanoids is kept to a minimum by keeping the tissue cold during mucosal separation and by homogenizing in acid ethanol to inhibit enzyme activity (Bennett *et al.*, 1973). This has an advantage over methods that allow the formation of prostaglandins during the strong stimulus of tissue processing, probably with the simultaneous dilution of the drug. We know that drugs can increase (Berstock *et al.*, 1980) or decrease (present results with aspirin) the amounts of prostaglandins extracted in this way.

Our *ex vivo* results with carbenoxolone differ from previously published *in vitro* data showing that this drug increased the gastric PGE yield. Martin *et al.* (1983) incubated fragments of rat stomach, including muscle as well as mucosa, with a very high concentration of carbenoxolone (2.5 mg ml^{-1}, about 4 mM). The experiments of Peskar & Weiler (1983) with human gastric mucosa *in vitro* also used high concentrations (0.4 and 1.6 mM, about 0.25–1 mg ml^{-1}). It is not possible to determine how much carbenoxolone reaches its site of anti-ulcer action within the mucosa and how much nonspecific binding occurs but these concentrations seem excessive. In ulcer patients given 100 mg carbenoxolone 3 times daily for a week the gastric ulcer rim contained 25.5 ± 2.6 (s.e.mean) μg carbenoxolone g^{-1} tissue, and serum levels were 65.9 ± 19.6 μg ml^{-1} (Peskar, 1980). Nevertheless, these amounts may be sufficient *in vivo* to affect the prostanoid content of human gastric mucosa since Rask-Madsen *et al.* (1983) found that carbenoxolone increased the amount of PGE_2 in the gastric juice of patients given carbenoxolone. A major question is whether the different findings depend only *in vitro* as opposed to *in vivo* studies, or whether there is a species

difference. The latter may well be the case, since carbenoxolone 19–1000 $\mu g\,ml^{-1}$ had little or no effect on the amount of 6-keto-$PGF_{1\alpha}$ or TXB_2 in incubates of rat isolated gastric corpus mucosa, although concentrations of 56–1000 $\mu g\,ml^{-1}$ increased the amount of PGE_2 and decreased the amount of the 15-keto-13,14-dihydro metabolite (B.M. Peskar, personal communication). There are other variations between tissues and species. In phagocytosing rat peritoneal leucocytes, carbenoxolone produced, if anything, a reduction in the yield of PGE_2-like material (Capasso *et al.*, 1983). However, in rabbit isolated kidney medulla, carbenoxolone 0.1–0.5 mM increased the amount of PGE-like material, while a decrease occurred with 1–5 mM (Vapaatalo *et al.*, 1978).

Our failure to alter the prostanoids in rat stomach *ex vivo* occurred despite the ability of other treatments to alter prostaglandin yields. The amounts were reduced by aspirin, and we previously found that the cytotoxic drugs melphalan and methotrexate increased the gastric formation of prostaglandin-like material (Berstock *et al.*, 1980). We therefore think that prostanoids are unlikely to play an important role in the anti-ulcer activity of carbenoxolone and DGL in the rat. Even though prostaglandins may be involved in the action of carbenoxolone in man, various other mechanisms may also participate such as alterations of cell turnover and stimulation of mucus secretion (Klein *et al.*, 1975). Similar actions also occur in the rat, and may explain the anti-ulcer effect of carbenoxolone and DGL (see Klein *et al.*, 1975; Van Marle *et al.*, 1981).

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