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COMMUNICATIONS

Effect of carbenoxolone on prostaglandin synthesis and degradation

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Although carbenoxolone has been shown to cause various biochemical changes in gastric mucosa (Lancet, Editorial, 1975), its mechanism of action remains unclear. We have now investigated the influence of the drug on the enzymes of prostaglandin (PG) synthesis and degradation. Several PGs and PG analogues are potent antisecretory and anti-ulcer agents (Robert, Nezamis & Philips, 1968; Karim, Carter & others, 1973; Carter, Ganesan & others, 1974). Furthermore, a number of PGs, irrespective of whether they are antisecretory, seem to exert a cytoprotective effect on gastric mucosa (Robert, 1975). Finally, an endogenous PGE_2-like substance (Bennett, Stamford & Unger, 1973) and PG metabolizing enzymes (Peskar & Peskar, 1975, 1976) have been demonstrated to occur in human gastric mucosa.

Guinea-pigs (300–600 g) were killed by a blow on the head. Their lungs and kidneys were excised and each homogenized in 30 ml of ice-cold 0·05 M potassium phosphate buffer, pH 7·4. After centrifugation at 10 000 *g* at 4° for 15 min the supernatants were re-centrifuged at 100 000 *g* at 4° for 1 h. The clear supernatant of guinea-pig lung (protein concentration 7·5–12·0 mg ml^{-1}) was used as enzyme source for 15-hydroxy-PG-dehydrogenase, Δ^{13}-reductase and histamine *N*-methyltransferase. The 100 000 *g* pellet of guinea-pig kidney was washed with buffer and re-centrifuged. It then was taken up and homogenized in 1·5 ml of 0·1 M potassium phosphate buffer containing 5 mM adrenaline and 5 mM glutathione. The resulting suspension (protein concentration 17–27 mg ml^{-1}) was used as PG-synthetase. Protein was determined by the method of Lowry, Rosebrough & others (1951).

Small biopsy specimens (20–50 mg total wet weight) of human gastric mucosa were obtained from patients undergoing gastroscopy for diagnostic purposes. The tissue samples were homogenized and the enzymes 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase determined in the 100 000 *g* supernatant as described previously (Peskar & Peskar, 1975, 1976).

15-Hydroxy-PG-dehydrogenase activity was determined by incubation of enzyme, 1 mM NAD as coenzyme and 100 ng per tube of PGE_2 or $PGF_{2\alpha}$ as substrate in a total volume of 0·19 ml at 37° for 5 min. The reaction was stopped by incubation in a boiling water bath for 1 min. Δ^{13}-Reductase activity was determined similarly except that 1 mM NADH (guinea-pig lung) or 15 mM NADH (human gastric mucosa) as coenzyme and 100 ng per tube of either 15-keto-PGE_2 or 15-keto-$PGF_{2\alpha}$ as substrate were used. Incubation time for Δ^{13}-reductase was 20 min for human gastric mucosa and 10 min for guinea-pig lung. PG-synthetase was measured by incubation of enzyme from guinea-pig kidney with 10 μg per tube arachidonic acid (Serva Feinbiochemica, Heidelberg, West Germany) at 37° for 10 min. Histamine *N*-methyltransferase was determined radiometrically exactly as described by Axelrod & Vesell (1970). Incubation time was 60 min. Preliminary experiments had shown that with the incubation conditions described all enzyme reactions proceeded linearly for the incubation times used. Specific activities of the enzymes (mean $\pm$ s.e.m., n = 3–6)

were 24·4 ± 2·6 ng mg^{-1} protein min^{-1} for 15-hydroxy-PG-dehydrogenase (measured as $PGF_{2\alpha}$ metabolized), 15·50 ± 5·49 ng mg^{-1} protein min^{-1} for Δ^{13}-reductase (measured as 13,14-dihydro-15-keto-$PGF_{2\alpha}$ synthesized from 15-keto-$PGF_{2\alpha}$), 4·51 ± 0·59 ng PGE_2 plus 7·18 ± 0·57 ng $PGF_{2\alpha}$ synthesized mg^{-1} protein min^{-1} for PG-synthetase and 4·26 ± 0·79 nmol *N*-methylhistamine formed mg^{-1} protein 60 min^{-1} for histamine *N*-methyltransferase. Indomethacin (1 mg ml^{-1}) (Merck, Sharp & Dohme) was dissolved in 0·1 M sodium phosphate buffer, pH 7·4. Carbenoxolone sodium was dissolved by freshly preparing a 2% solution in water and diluting to 1 mg ml^{-1} with 0·1 M sodium phosphate buffer, pH 7·4. All further dilutions of drugs were made with the same buffer, and 50 μl was added to the incubation mixtures. Control tubes contained 50 μl of the buffer.

After stopping the enzyme reactions the incubation mixtures were diluted to 4·0 ml with water and centrifuged (3000 rev min^{-1}, 10 min). Concentrations of products and remaining substrates in the supernatants were determined by radioimmunoassays. The specificities and sensitivities of the radioimmunoassays for PGE_2, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-PGE_2 have been described previously (Peskar & Hertting, 1973; Liebig, Bernauer & Peskar, 1974; Peskar & Peskar, 1976). None of the drugs used interfered non-specifically with the radioimmunoassays.

Fig. 1 shows that carbenoxolone in a dose-dependent manner inhibits oxidation of $PGF_{2\alpha}$ by 15-hydroxy-PG-dehydrogenase, and reduction of 15-keto-$PGF_{2\alpha}$ by Δ^{13}-reductase of guinea-pig lung. The ID50 for both reactions was about 3×10^{-5} M and a concentration of 4×10^{-4} M caused 100% inhibition. Fig. 1 also shows that carbenoxolone in the same dose range did not influence histamine *N*-methyltransferase, indicating selectivity for the inhibition of the PG-metabolizing enzymes. Comparable results were obtained when PGE_2 and 15-keto-PGE_2 were used instead of $PGF_{2\alpha}$ and 15-keto-$PGF_{2\alpha}$ as substrates for 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase of guinea-pig lung. Indomethacin in high concentrations inhibits both 15-hydroxy-

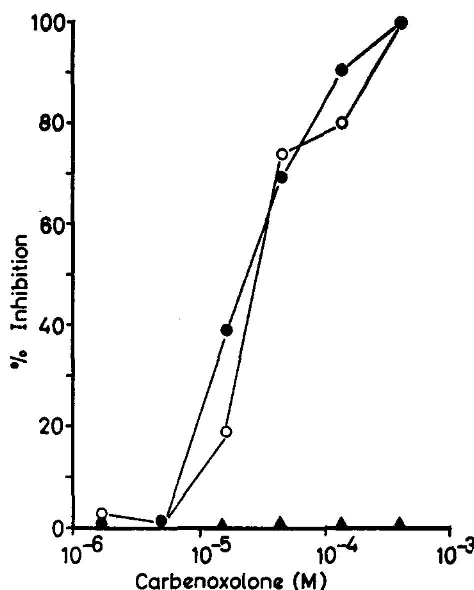

FIG. 1. Effect of carbenoxolone on three enzymes in the 100 000 *g* supernatant of guinea-pig lung. 15-hydroxy-prostaglandin-dehydrogenase (○), Δ^{13}-reductase (●), histamine *N*-methyltransferase (▲). Prostaglandin $F_{2\alpha}$ and 15-keto-prostaglandin $F_{2\alpha}$ were used as substrates for the dehydrogenase and reductase respectively.

PG-dehydrogenase (Flower, 1974) and Δ^{13}-reductase (Pace-Asciak & Cole, 1975; Peskar & Peskar, 1976); carbenoxolone on a molar basis was almost three times more effective in inhibiting both enzymes. Finally, considering the pharmacological activity of carbenoxolone as an anti-ulcer agent, it is remarkable that carbenoxolone in the dose range shown in Fig. 1 not only inhibited the PG metabolizing enzymes of guinea-pig lung but also 15-hydroxy-PG-dehydrogenase and Δ^{13}-reductase of human gastric mucosa.

Indomethacin, a potent inhibitor of PG synthesis (Vane 1971), prevents formation of PGE_2 and $PGF_{2\alpha}$ from arachidonic acid to about the same degree (Flower, Cheung & Cushman, 1973). We found an ID50 of 4×10^{-6} M for the anti PG-synthetase activity of indomethacin in guinea-pig kidney; carbenoxolone at the same dose was ineffective but at 4×10^{-5} M inhibited $PGF_{2\alpha}$ synthesis by 50%, while synthesis of PGE_2 was unaffected. Inhibition of synthesis with the highest concentration of carbenoxolone employed (4×10^{-4} M) was almost 80% for $PGF_{2\alpha}$, but less than 15% for PGE_2.

The concentrations of carbenoxolone necessary for enzyme inhibition are relatively high, but such concentrations might be reached in gastric mucosa during absorption of the drug. Downer, Galloway & others (1970) in experiments on man have shown that absorption of orally administered carbenoxolone is rapid and dependent on the pH of gastric contents. They concluded that the stomach is the major site of absorption of carbenoxolone, but it remains to be determined if the effects of carbenoxolone demonstrated *in vitro* on the enzymes of PG synthesis and degradation also occur *in vivo* and contribute to the pharmacological activity of the drug.

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