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# The effect of sodium carbenoxolone on lysosomal enzyme release

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Lysosomes contain a multitude of acid hydrolases capable of breaking down cellular macromolecules, including glycoproteins. Lysosomes exist in most animal tissues including the stomach [1], which is protected by a layer of gastric mucus consisting mainly of glycoproteins [2]. The protective role of this mucus and therefore the development of gastric lesions is partly dependent on the controlled activity of gastric lysosomes. Ferguson *et al.* [3] found that gastric ulcers in restrained rats were associated with reduced mucosal levels of cathepsin D, indicating rupture of lysosomal membranes and release of enzymic contents. The action of these enzymes on the mucus lining the stomach could be responsible for the 'underglycosylated' glycoprotein apparent in gastric lesions. Similar evidence exists for lysosomal involvement in drug induced gastric ulceration. 5-Hydroxytryptamine was found to induce gastric ulcers in rats, associated with a release of lysosomal enzymes [4], and the non-steroidal antiinflammatory drug phenylbutazone, at ulcerogenic doses, was found to accelerate the breakdown of lysosomes in isolated stomach preparations from the rat and rabbit [5]. The present study was designed to examine the effects of the anti-ulcerogenic drug carbenoxolone on the stability and enzyme release from lysosomes, in order to establish the possible sub-cellular modes of action of the drug.

## MATERIALS AND METHODS

Phenolphthalein glucuronide, *p*-nitrophenyl phosphate, *p*-nitrophenyl-*N*-acetyl-β-D-glucopyranoside and Triton X-100 were obtained from the Sigma Chemical Company Ltd., London. Carbenoxolone sodium was a gift from Biorex Laboratories Ltd., London.

A lysosome fraction was isolated using the method of Symons *et al.* [6]. Male Wistar albino rats (body weight approx. 300 g) were used. Animals were killed by cervical dislocation, livers removed, weighed and placed in ice-cold 0.25 M sucrose. The liver was then chopped finely with scissors (2–3 mm cubes) and portions homogenised in 0.25 M sucrose using a Potter Elvejhëm homogeniser. All operations were carried out at 4°. This initial suspension was diluted to a final concentration of 10% w/v with 0.25 M sucrose and centrifuged at 750 $g_{av}$ for 10 min to sediment unbroken cells, debris and nuclei. The supernatant was then centrifuged at 20,000 $g_{av}$ for 20 min to sediment a 'large granule' fraction containing unbroken lysosomes. The pellet was then washed twice in 0.25 M sucrose and finally suspended in 0.25 M sucrose containing 0.05 M Tris–HCl buffer (pH 7.4). 2 ml of sucrose-buffer were added for every g of liver used. Portions (5 ml) of the lysosome suspension were added to 50 ml conical flasks, stoppered and shaken in a 37° water bath at 100 oscillations per min for 90 min. Various concentrations of carbenoxolone were added in small volumes of water (10–20 μl) to give a final concentration of $10^{-8}$–$10^{-3}$ M. Appropriate controls were always run. The level of free enzymes in lysosomal suspensions before incubation was measured by centrifuging a small volume (5 ml) at 20,000 $g_{av}$ for 20 min and assaying enzyme activity in the supernatant. In a similar way the free enzyme levels present after control and experimental incubations were also measured. The total enzyme releasable from the lysosome preparation was taken as that released after 90 min incubation in the presence of 0.1% v/v Triton X-100.

Acid phosphatase (orthophosphoric monoester phosphohydrolase) was measured using the method of

Huggins and Talahay [7]: β-glucuronidase (β-D glucuronide glucuronohydrolase) by the method of Talahay *et al.* [8] and *N*-acetyl glucosaminidase (β-2-acetimido-2-deoxy-D-glucoside acetamidodeoxy-glucohydrolase) by the method of Caygill and Jevons [9].

## RESULTS AND DISCUSSION

The release of acid phosphatase, β-glucuronidase and *N*-acetyl glucosaminidase caused by sodium carbenoxolone over a concentration range of $10^{-8}$ to $10^{-3}$ M is shown in Fig. 1. All results have been expressed as a percentage of control release from the lysosomal suspension over the 90 min period. It can be seen that carbenoxolone appeared to have a biphasic effect. Lysis of the lysosomes leading to increased enzyme leakage occurred with concentrations of $10^{-4}$ M and above while lower concentrations showed either a stabilizing effect or no significant effect at all. Maximum stabilization occurred with all enzymes at $10^{-5}$ M carbenoxolone. The degree of stabilization was between 12 and 22 per cent. The maximum lytic effect of carbenoxolone at $10^{-3}$ M was very much greater than the maximum stabilization. Values for acid phosphatase, β-glucuronidase and *N*-acetylglucosaminidase release were 736, 680 and 439 per cent of controls respectively. Incubation with 0.1% v/v Triton X-100 released 1152, 932 and 984 per cent for the same three enzymes. Control incubations, over the 90 min period, therefore released 8.7 per cent of total acid phosphatase, 10.7 per cent of total β-glucuronidase and 10.2 per cent of total *N*-acetyl glucosaminidase.

These studies show that concentration is of critical importance when considering the effects of carbenoxolone on lysosomes. Lysosomal fragility is important in the genesis of drug induced ulceration in animals and possibly in chronic gastric ulceration in humans. Acute alcohol intoxication which often causes gastric lesions is associated with abnormal fragility of lysosomes and discharge of enzymes [10]. Ulceration of the caecum in guinea pigs by carrageenan has been attributed to lysis of macrophage lysosomes [11]. In contrast to these results prostaglandin $E_1$ which has been shown to have an anti-ulcerogenic effect [12] has been shown to decrease the leakage of lysosomal enzymes in the gastric mucosa [4]. It is not clear at present whether release of lysosomal enzymes is causative in the production of a lesion or merely an indication of tissue damage. However, even if the latter, the release of lysosomal enzymes with their considerable digestive capabilities would aid the development of a lesion. The results presented in this paper indicate that at certain concentrations carbenoxolone *in vitro* stabilizes lysosomal membranes. Such an effect on gastric mucosal lysosomes could explain the anti-ulcerogenic action of this drug. The membrane stabilizing role of carbenoxolone is supported by the work of Symons [13] who found that $10^{-6}$ M concentrations of the drug resulted in decreased permeability of phosphatidylcholine and phosphatidylcholine: cholesterol liposomes.

The lytic effect of carbenoxolone demonstrated in these studies is probably of no pharmacological significance. Carbenoxolone is rapidly absorbed and has a high degree of binding to plasma proteins [14]. Two hours after a therapeutic dose of 100 mg in man plasma levels are approximately 15 μg/ml [15]. It is unlikely therefore that the drug would reach lytic concentrations in the vicinity of the gastric mucosal lysosomes.

In conclusion it may be postulated that lysosomal fragility is important in the genesis of drug induced ulceration in animals and possibly in chronic gastric ulceration in man. Whether rupture of these organelles is a primary feature or secondary in aiding the development of the lesion, lysosomal stabilization could, in part, explain the beneficial action of carbenoxolone in the treatment of this disease. Such a stabilization could explain the decreased cell turnover noted by Lipkin [16] and may explain the increase in glycoprotein synthesis which occurs after carbenoxolone treatment [17].

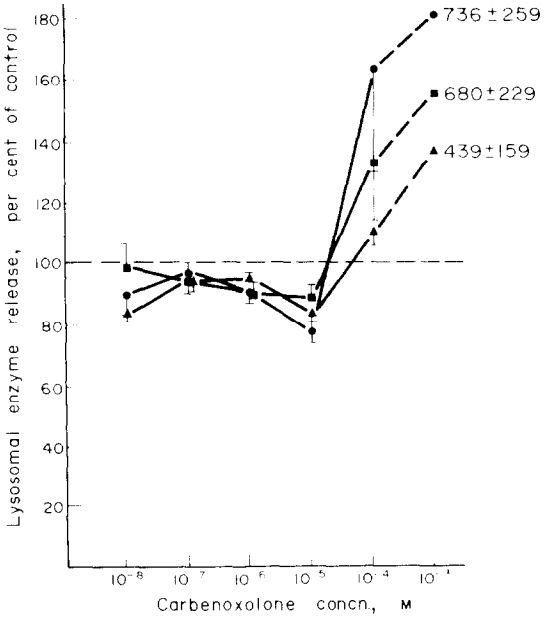


Fig. 1. The effect of various concentrations of sodium carbenoxolone on lysosomal enzyme release. ●——●, acid phosphatase; ■——■, β-glucuronidase; ▲——▲, *N*-acetyl glucosaminidase. Results are shown as means ±S.E.M.'s of four experiments.

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# Rat liver aldehyde dehydrogenase—Immunochemical identity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inducible normal liver and 2-acetylaminofluorene inducible hepatoma isozymes*

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Interest in mammalian aldehyde dehydrogenases (EC 1.2.1.3 to 1.2.1.5, ALDH) has centered on the role of this enzyme system in ethanol metabolism[1–4]. A number of molecular forms of aldehyde dehydrogenase have been demonstrated in the mitochondrial, microsomal and cytosolic fractions of rat liver and several other mammalian tissues[5–10]. However, it is generally agreed that only certain of the liver and perhaps brain mitochondrial ALDH isozymes are responsible for the oxidation of acetaldehyde during ethanol metabolism[1–4]. To date, the role(s) of the microsomal and cytosolic aldehyde dehydrogenases remain largely undefined.

Evidence for multiple molecular forms of cytosolic aldehyde dehydrogenase comes from a variety of studies[5–14]. Among these various ALDH isozymes, Deitrich *et al.*[14, 15] have demonstrated that phenobarbital increases liver cytosolic ALDH activity 10- to 30-fold in certain genetically defined lines of rat. The increased activity is due to a phenobarbital-inducible ALDH ($\phi$ isozyme), which differs in a variety of properties from the basal, non-inducible ALDH[14, 15]. Recently, another cytosolic ALDH, inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) ($\tau$ isozyme), has been purified from rat liver[16, 17]. The $\tau$ isozyme is physically, functionally and immunochemically distinguishable from the $\phi$ isozyme. In addition, Feinstein *et al.*[12, 18, 19] and Lindahl[20] have reported the presence of several cytosolic ALDH isozymes unique to hepatomas induced in several strains of rat by a variety of chemical carcinogens, including 2-acetylaminofluorene (2-AAF) and dimethylaminoazobenzene (DAB). This series of hepatoma-specific aldehyde dehydrogenases ($\alpha$ isozymes) has also been purified and immunochemically characterized[19].

The kinetic properties of these inducible aldehyde dehydrogenases (mM $K_m$ values for acetaldehyde) make it unlikely that any of the three isozymes, $\phi$, $\tau$ or $\alpha$, plays a major role in ethanol metabolism. More probable roles for these isozymes are in the metabolism of other aldehydes, both biogenically generated and exogenously administered. Work in our laboratories has been directed toward determining the functional significance of these various inducible ALDH. To this end, we have recently become interested in determining the functional, structural and genetic relationships among the $\phi$, $\tau$ and $\alpha$ isozymes. Here we report the results of a direct immunochemical comparison of these ALDH, each of which is induced by different conditions.

Rabbit antiserum against rat hepatoma-specific aldehyde dehydrogenase was produced as described previously[19]. Rabbit antiserum against $\tau$ isozyme was produced by a hyperimmune protocol (M. Roper *et al.*, manuscript in preparation). Ouchterlony 2-dimensional immunodiffusions were also performed as described[19]. The antiserum generated against purified $\alpha$ isozymes possesses two distinct antibody populations[9, 20]. One antibody population forms enzymatically active immune complexes and is hepatoma-specific, whereas the other population forms enzymatically inactive immune

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