Short communications

Effects of carbenoxolone sodium on stress-induced gastric damage in rats

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Carbenoxolone sodium was studied to determine its effect against restraint-induced gastric mucosal damage in the rat. Significant anti-ulcer activity was observed following intraperitoneal administration in non-fasted but not in either 24 or 48 h fasted rats. Orally, carbenoxolone had no statistically significant effect in non-fasted rats although the data suggest that it may have afforded some protection. These results indicate that the use of non-fasted rats is most probably a requirement for demonstrating the protective activity of this type of anti-ulcer compound in stressed rat assays.

Several actions may account for the anti-ulcer activity of carbenoxolone. It has been shown to reduce gastric acid output in response to histamine in man (Bank, Marks, Palmer, Groll & Van Eldik, 1967; Cocking & MacCaig, 1969) and to reduce the total acidity of gastric juice collected from anaesthetized pylorus ligated rats (Henman, 1970). In addition to an effect on gastric acidity, Henman (1970) has demonstrated dose-related antipeptic activity with this compound both *in vivo* and *in vitro* and suggests that its therapeutic activity may be related in part to its effect on gastric acidity and peptic activity. A mucigogue effect may be important since histological data indicate that carbenoxolone enhances gastric mucus secretion (Goodier, Horwich & Galloway, 1967; Dean, 1967; Lipkin & Ludwig, 1967; Johnson, 1967). Thus, carbenoxolone may be unique in that it may facilitate ulcer healing by simultaneously decreasing the digestive capacity of gastric juice and increasing mucosal resistance.

Little has been published regarding the effect of carbenoxolone in standard animal ulcer tests. Pretreatment with carbenoxolone reduces the ulcerogenic effect of compound 48/80 in rats (Dean, 1967) and the susceptibility of guinea-pigs to 24 h, but not 18 h, resistance-stress-induced gastric erosions (Lipkin & Ludwig, 1967). Also, Khan & Sullivan (1967) demonstrated that carbenoxolone increases the rate of healing of electrocautery-induced gastric ulcers in the rat. These authors note that it has not been possible to demonstrate a protective effect of carbenoxolone against restraint- or 5-hydroxytryptamine-induced ulceration in the rat. The purpose of this communication is to present results of preliminary experiments which indicate that carbenoxolone sodium will reduce the incidence of stress-induced gastric glandular mucosal damage in the rat.

Methods.—Acute restraint stress was employed to induce gastric glandular mucosal damage in non-fasted and 24 or 48 h fasted Charles River CD strain female rats weighing 80–120 grammes. Fasted animals were deprived of rat chow for 24 or 48 h but had access to two sugar cubes and water. Each rat was anaesthetized with thiopentone sodium (40 mg/kg i.p.), drug or carrier was administered, and a plaster of paris bandage was applied over gauze to form a closely fitting cocoon (Sines, 1959) from which only the head protruded. After 20–24 h, the animals were killed by cervical dislocation and the cocoons removed. The abdomen of each rat was opened, the pylorus clamped, the stomach distended with saline (0·9% w/v NaCl solution) via an oral tube, the oesophagus clamped, and the stomach excised. The stomachs were placed in a 0·4% formaldehyde solution for approximately 30 s to harden the outer layers and facilitate examination (Brodie & Hanson, 1960). Each stomach was then cut open along the greater curvature and the number of gastric erosions in the glandular portion (corpus and antrum) recorded. The Mann-Whitney-Wilcoxon rank sum test was used to compare the median number of gastric erosions in the control and treated groups to determine statistical significance. Carbenoxolone sodium (synthesized by Dr. R. Nelson, Medicinal Chemistry Department) was dissolved in distilled water.

Results.—The results of these experiments are summarized in Tables 1a and b. Intraperitoneally administered carbenoxolone (40 mg/kg) had no effect on stress-induced damage in either 24 or 48 h fasted rats (Table 1a). In non-fasted animals, in contrast, carbenoxolone caused a signi-

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TABLE 1a. *Effects of intraperitoneal carbenoxolone (40 mg/kg) on restraint-induced gastric damage in fasted and non-fasted rats*

	Experiment No.	No. of animals Control	Carb.	% Animals with gastric glandular mucosal damage Control	Carb.	Total No. of lesions Control	Carb.	% Change in No. of lesions	*P*
Non-fasted	1	13	12	61·5	25	33	3	−90·9	0·045
	2	14	11	78·5	18	42	4	−90·4	0·003
	3	11	10	72	40	33	16	−51·5	0·212
24 h Fast	4	10	9	80	88	62	48	−22·5	>0·5
48 h Fast	5	14	13	43	69	14	39	+178·5	0·133
	6	11	12	72	50	20	17	−15·0	>0·5
	7	12	9	91	88	97	76	−21·6	>0·5

TABLE 1b. *Effects of intraperitoneal and oral carbenoxolone on restraint-induced gastric glandular mucosal damage in non-fasted rats*

Experiment No.	Dose (mg/kg)	Route	No. of animals Control	Carb.	% Animals with gastric damage Control	Carb.	Total No. of lesions Control	Carb.	% Change in No. of lesions	*P*
1	10	i.p.	8	10	75	25	35	46	+31·4	>0·5
2	20	i.p.	9	9	78	22	26	5	−80·7	0·02
1	40	i.p.	8	10	75	20	35	4	−88·5	0·01
2	40	i.p.	9	8	78	13	26	3	−88·4	0·014
3	160	oral	11	10	82	50	32	12	−62·5	0·11
4	160	oral	9	9	89	100	56	23	−58·9	>0·5

ficant ($P<0·05$) decrease in the number of stress-induced gastric lesions in 4 of 5 experiments at 40 mg/kg i.p. (Tables 1a and b) and in one experiment at 20 mg/kg i.p. (Table 1b). No anti-ulcer activity was observed in non-fasted animals at the 10 mg/kg i.p. dose (Table 1b). In non-fasted animals, carbenoxolone (160 mg/kg) given orally had no statistically significant effect against restraint-induced damage although in one experiment a 62·5 and in the other a 58·9% reduction in the total number of lesions was observed (Table 1b).

Discussion.—It has been possible to demonstrate a protective effect of carbenoxolone sodium against stress-induced gastric glandular mucosal damage in rats. Significant anti-ulcer activity was observed with this drug in non-fasted but not in either 24 or 48 h fasted rats. The use of non-fasted rats is most probably a requirement for demonstrating the anti-ulcer activity of this type of compound in stressed rat assays.

No reason can be given for the difference in activity in fasted and non-fasted rats. Fasting itself is a stress and will increase the effect of other stressors (Selye, 1950). Brodie & Hanson (1960) found that starvation (i.e., total food deprivation) increases the severity and incidence of restraint-induced gastric damage in the rat. No such effect was observed in our study, possibly because fasted rather than starved rats were used. However, it still may be that carbenoxolone is not sufficiently potent to overcome the ulcerogenic effect of synergistic stresses. Likewise, fasting may block the suggested mucigogue activity (Goodier, *et al.*, 1967; Dean, 1967; Lipkin & Ludwig, 1967; Johnson, 1967) of this compound and, therefore, its anti-ulcer activity. This seems unlikely, however, as the data presented by Robert, Boyer & Nezamis (1963) indicate that fasting for up to 48 h does not depress the synthesis or release of gastric mucus in the rat. The possibility exists that the anti-ulcer and mucigogue activities of this compound are not directly related.

Previous studies (Dean, 1967; Lipkin & Ludwig, 1967) have demonstrated anti-ulcer activity following pretreatment with carbenoxolone. In our study, significant anti-ulcer activity was observed following intraperitoneal but not oral administration when the compound was administered immediately before stressing. However, the data suggest that orally administered car-

benoxolone may have afforded some protection. In each of two experiments, the total number of gastric lesions was reduced by more than fifty per cent. Inability to demonstrate a significant oral anti-ulcer effect may be related to the way carbenoxolone is metabolized in the rat. Parke (1967) found that one-half to two-thirds of an oral dose of carbenoxolone is hydrolyzed to glycyrrhetinic acid and succinate in the rat gastrointestinal tract before primary absorption. Assuming carbenoxolone is the active substance, significant oral activity against restraint-induced ulcerogenesis should result on increasing the dose of drug.

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