and the increase of AUC. The decrease of the total clearance can be, in part, attributed to the decrease in the volume of distribution. But the total clearance comprises not only renal excretion but all other pathways of elimination including drug metabolism. Diabetes mellitus involves disorders of many metabolic pathways. The hypothesis of a diabetic-induced decrease of the nitroxide reduction cannot be discarded.

In summary, the initial increase of blood concentration of free radical is insufficient to explain the MRI abnormalities observed since elimination rates are rapid and similar both in control and diabetic rats. The prolongation of contrast visualization in the kidney might be explained by a stasis of contrast media in the kidney secondary to the tubular nephropathy and, or, a decrease of renal metabolism leading to a persistance of paramagnetism.

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The protective mechanisms of paracetamol against ethanol-induced gastric mucosal damage in rats

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Abstract—The protective mechanisms of paracetamol against ethanol-induced gastric mucosal damage have been examined. The antiulcer action of subcutaneously (s.c.)-injected paracetamol, 250 mg kg^{-1}, was attenuated by either subdiaphragmatic vagotomy or s.c. injection of *N*-ethylmaleimide, 10 mg kg^{-1}. This attenuation was not seen in rats given paracetamol by the oral route (p.o.). Indomethacin pretreatment, 5 mg kg^{-1}, did not influence the lesion-preventing action of paracetamol given s.c. or p.o. The findings suggest that the antiulcer effect of s.c.-administered paracetamol results from an action involving the vagal nerve and tissue sulfhydryls, but not prostaglandins. On the other hand, the protective mechanism of paracetamol p.o. is independent of the vagal system or tissue sulfhydryls and prostaglandins. It seems that paracetamol given p.o. exerts its antiulcer effect by acting directly on the mucosal cell to strengthen mucosal integrity.

Paracetamol when given orally (p.o.) reduces ethanol-induced gastric mucosal damage (Poon et al 1988). As subcutaneously (s.c.)-injected paracetamol also effectively antagonizes ethanol-induced gastric lesions (Poon et al 1988), it is possible that the protective mechanism could be mediated, partly or wholly, through a systemic pathway. Somasundaram & Ganguly (1987) have shown the importance of the vagus nerve in maintaining normal levels of gastric mucus glycoproteins and, thus, mucosal integrity; absence of vagal influence weakens the mucus barrier. It is, therefore, reasonable to postulate that the systemic action of paracetamol may be mediated through the vagal nerve.

Sulfhydryls and prostaglandins protect against ethanol-induced mucosal damage (Szabo 1986; Konturek et al 1987a). As paracetamol has been shown to stimulate prostaglandin production in the gastric wall (Van Kolfschoten & Van Noordwijk 1987), it has been suggested that these eicosanoids may participate in the lesion-antagonizing mechanism of the drug. The role of sulfhydryls and prostaglandins in the antiulcer action of paracetamol is, however, still unclear.

The present study examines the role of the vagus, sulfhydryls and prostaglandins in relation to the protective action of paracetamol against ethanol-induced gastric mucosal damage in rats.

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Materials and methods

Male Sprague-Dawley rats were fed a standard laboratory pellet diet (Ralston Purina Co.) and drank tap water. Food was withheld 24 or 48 h before the animals were used, depending on the type of experiment; the rats were kept in cages with wide wire mesh floors to prevent coprophagy and allowed free access to tap water which was removed 1 h before starting experiments. All experiments were conducted in an air-conditioned room (temperature 22 ± 1°C, relative humidity 65–70%) where the animals were normally housed. The rats were killed, by a sharp blow on the head, immediately at the end of each experiment and their stomachs removed. The areas of the lesions in the glandular segment of the stomach were measured with a grid (each grid was 1 mm^2) (Ogle et al 1985). In the case of petechiae, five such lesions were taken as the equivalent of 1 mm^2. The sum of the lesion areas in each group was divided by the number of animals and expressed as the mean lesion index.

Experiment 1. Rats (180 ± 20 g) were subjected to subdiaphragmatic vagotomy under light ether anaesthesia. The upper abdominal cavity was opened by a midline incision beneath the sternum. The anterior and posterior branches of the vagal nerve,

lying on the surface of the oesophageal end of the stomach, were identified and severed. Phenol (Sigma) solution (1 M) was then painted around the oesophageal end of the stomach to ensure completeness of vagotomy. After 5 s, the phenol was washed off by gentle lavage with physiological saline solution (NaCl in distilled water, 0·9% w/v). The abdominal wall and skin were sutured after the operation. Animals acting as controls were similarly operated upon, except that their vagus nerves were left intact (sham operated). During the postoperative period, all the rats were fed a normal diet and drank tap water. Four days later, food, but not water, was withheld for 48 h, then paracetamol (Sigma), 62·5, 125 or 250 mg kg^{-1}, suspended in Tween 80 (Sigma) 4% v/v, was given to the animals either by s.c. injection, 5 mL kg^{-1}, or p.o. via a gastric tube, 5 mL kg^{-1}. Ethanol (Merck) 40% v/v was prepared in distilled water, and given p.o. in a volume of 10 mL kg^{-1}. Appropriate volumes of the vehicles for paracetamol or ethanol were given by the same route to the controls. All animals were killed 1 h after ethanol administration. The lesion index of the glandular stomach was then assessed.

Experiment 2. Rats (200 ± 20 g) had no food for 24 h before but free access to tap water up to 1 h before the s.c. or p.o. administration of paracetamol. Ten min after paracetamol treatment, *N*-ethylmaleimide (NEM) (Sigma), dissolved in saline, was injected s.c. in a dose of 10 mg kg^{-1}. Ethanol 40%, 10 mL kg^{-1}, was then given orally to the rats 20 min after NEM. The animals were killed 1 h after ethanol treatment and the gastric glandular lesion index evaluated.

Experiment 3. Rats (200 ± 20 g) had no food for 24 h, but free access to tap water up to 1 h before indomethacin (Sigma), 5 mg kg^{-1}, dissolved in absolute ethanol, was injected s.c. 1 h before the administration of paracetamol; controls were given the alcohol vehicle, in a similar volume (1 mL kg^{-1}). Thirty min after paracetamol administration, ethanol 40%, 10 mL kg^{-1}, was given p.o. The animals were then killed 1 h later and the lesion areas in the stomach measured.

Results

Subdiaphragmatic vagotomy did not significantly influence ethanol-induced gastric damage, the lesion index being similar to that of the sham-operated controls (Table 1). Pretreatment with paracetamol s.c. or p.o. dose-dependently prevented ethanol-induced erosions in sham-operated rats. The effect of subdiaphragmatic vagotomy on the antiulcer action of paracetamol given s.c. or p.o. was different. When the drug was given s.c., its protective effect was attenuated (Table 1A), the changes not being statistically different from the control. In contrast, oral administration of paracetamol continued to antagonize significantly ethanol-induced mucosal damage in vagotomized animals (Table 1B); the effect was dose-dependent.

Table 1. Effect of subdiaphragmatic vagotomy on the antiulcer action of paracetamol in rats given ethanol 40% p.o.

Treatment		Lesion index (mm^2) Sham	Vagotomy
A. s.c.			
Vehicle	5 mL kg^{-1}	20·1 ± 3·7 (11)	18·0 ± 3·2 (15)
Paracetamol	62·5 mg kg^{-1}	13·5 ± 3·5 (10)	16·1 ± 3·6 (12)
Paracetamol	125 mg kg^{-1}	10·2 ± 3·1 (10)	14·7 ± 4·9 (15)
Paracetamol	250 mg kg^{-1}	5·8 ± 2·1 (10)*	10·5 ± 2·8 (16)
B. p.o.			
Vehicle	5 mL kg^{-1}	24·2 ± 4·4 (10)	16·8 ± 3·1 (11)
Paracetamol	62·5 mg kg^{-1}	18·1 ± 3·2 (10)	12·0 ± 2·0 (10)
Paracetamol	125 mg kg^{-1}	16·0 ± 4·7 (12)	8·4 ± 2·3 (10)
Paracetamol	250 mg kg^{-1}	3·0 ± 1·3 (15)*	2·8 ± 1·3 (15)*

Vehicle: Tween 80 4%.
The values are the means ± s.e.m. The number of rats used in each group is indicated in parentheses.
*$P < 0·01$, compared with the corresponding operated group given vehicle.

Table 2. Effects of *N*-ethylmaleimide (NEM) treatment (10 mg kg^{-1}) s.c. on the antiulcer action of paracetamol in rats given ethanol 40% p.o.

Treatment		Lesion index (mm^2) Saline (2 mL kg^{-1})	NEM (10 mg kg^{-1})
A. s.c.			
Vehicle	5 mL kg^{-1}	14·4 ± 3·5	40·7 ± 9·8†
Paracetamol	250 mg kg^{-1}	5·6 ± 1·8*	44·1 ± 10·9†
B. p.o.			
Vehicle	5 mL kg^{-1}	9·4 ± 2·3	32·2 ± 7·7†
Paracetamol	250 mg kg^{-1}	0·6 ± 0·2*	0·8 ± 0·4**

Vehicle: Tween 80 4%.
The values are the means ± s.e.m. of 10 rats.
*$P < 0·05$; **$P < 0·01$, compared with the corresponding vehicle-treated control.
†$P < 0·01$, compared with the saline-injected group.

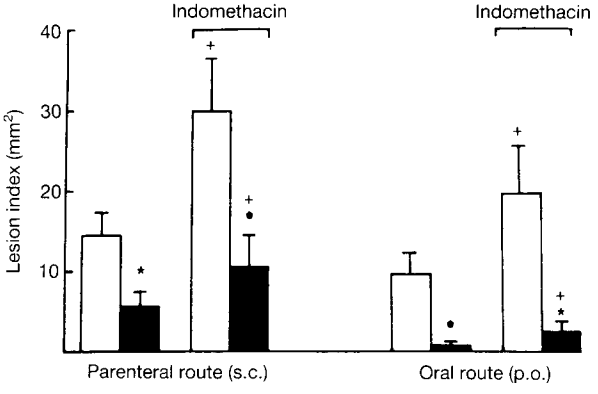

FIG. 1. Effect of treatment (1 h before paracetamol) with indomethacin, 5 mg kg^{-1} s.c., or its vehicle, 1 mL kg^{-1} s.c., on the antiulcer action of paracetamol in rats given ethanol 40% p.o. Vertical bars indicate s.e.m. (n = 10 in each group); open columns, vehicle (Tween 80 4%), 5 mL kg^{-1}; solid columns, paracetamol, 250 mg kg^{-1}. *$P < 0·05$, compared with its own vehicle-treated control; †$P < 0·05$, compared with the corresponding ethanol vehicle-injected group.

Table 2 shows the effect of NEM treatment on the antiulcer action of paracetamol in ethanol-treated animals. Injection of NEM, 10 mg kg^{-1} s.c., did not itself produce gastric lesions but it worsened ethanol-induced gastric damage. NEM, 10 mg kg^{-1}, given 10 min after paracetamol administration blocked the protective action of paracetamol only when the latter was given by the s.c. route.

Indomethacin, 5 mg kg^{-1}, worsened ethanol-induced mucosal damage, when compared to the controls receiving alcohol vehicle s.c. before Tween 80 s.c. or p.o. (Fig. 1). However, treatment with indomethacin 1 h before paracetamol administration did not influence the antiulcer effect of paracetamol given either s.c. or p.o. before lesion induction by ethanol.

Discussion

Vagal activity has been shown to be involved in ethanol-induced gastric damage (Henagan et al 1984), and the present finding that subdiaphragmatic vagotomy weakens the protective action

of paracetamol against mucosal damage (Table 1A), supports the notion that the vagus nerve may play an important role in the antiulcer property of paracetamol given by the s.c. route. The mechanism may be through vagal-induced alkaline secretion (Konturek et al 1987b) which is important for maintaining the integrity of the gastric mucosa. Endogenous sulfhydryls appear to be involved in gastric mucosal protection, because sulfhydryl-containing compounds are gastroprotective (Szabo 1986). Reduced tissue sulfhydryl levels are associated with increased gastric lesion severity induced by absolute ethanol (Konturek et al 1987a), indicating a close relationship between stomach wall sulfhydryls and ethanol-induced mucosal damage. The antiulcer action of paracetamol given s.c. could also be mediated via the action of sulfhydryls because the sulfhydryl-alkylator NEM abolished the protective action of paracetamol in ethanol-evoked gastric damage (Table 2A). One of the metabolic pathways of paracetamol is through cytochrome P450, leading to the release of an electrophilic metabolite which binds and depletes tissue glutathione, a major non-protein sulfhydryl. This pathological mechanism is unlikely because paracetamol 250 mg kg^{-1} used in the present study does not affect liver function, as reflected by normal serum glutamic-pyruvic transaminase activity (unpublished data). Furthermore, adverse hepatic effects are seen only with higher doses (Mitchell et al 1973; Jollow et al 1974).

When paracetamol was given orally, its antiulcer action was unaffected by vagotomy or sulfhydryl alkylation (Tables 1B, 2B), indicating that prevention of mucosal damage was probably independent of vagal activity and tissue sulfhydryls. The protective mechanism could, therefore, be through a direct action on the mucosal cells (Ota et al 1988). Prostaglandins are gastroprotective (Robert et al 1979), and paracetamol stimulates prostaglandin release in gastric mucosal tissue (Van Kolfschoten et al 1982). The current findings, however, do not support the possibility of prostaglandin involvement because the lesion-preventing effect of paracetamol was not reduced in animals which had been given indomethacin 1 h earlier; this treatment blocks prostaglandin synthesis effectively (Wallace et al 1988).

The findings in this study support the conclusion of Van Kolfschoten & Van Noordiwijk (1987) that the protective effect of paracetamol is not mediated through mucosal prostaglandins; they also point to the likelihood that the antiulcer action of s.c.- or p.o.-administered paracetamol is exerted through different mechanisms.

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