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Epidermal growth factor (EGF) in the gastroprotective and ulcer healing actions of colloidal bismuth subcitrate (De-Nol) in rats

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SUMMARY **Colloidal bismuth subcitrate (CBS; De-Nol) exhibits gastroprotective properties in experimental animals and enhances the healing of chronic gastroduodenal ulcers, but the mechanisms of these actions have not been entirely elucidated. The present study was designed to determine whether epidermal growth factor (EGF), which also has gastroprotective and ulcer healing properties, contributes to the action of De-Nol on the stomach in rats. It was found that De-Nol protects the gastric mucosa against ethanol damage and that this is accompanied by increased mucosal generation of prostaglandins (PG). Removal of the endogenous source of EGF (sialoadenectomy) did not significantly decrease the protective and PG stimulating effects of De-Nol. Pretreatment with exogenous EGF partially protected the stomach against ethanol injury, but did not influence the protective action of De-Nol in sialoadenectomised animals. De-Nol, like EGF given orally, enchanced the healing of chronic gastric and duodenal ulcers induced by serosal acetic acid. De-Nol was found to bind EGF in a pH-dependent manner and to accumulate it in ulcer area. Thus the peptide is available locally in high concentrations to accelerate the re-epithelialisation and tissue repair of the ulcerated mucosa. These ulcer healing effects of De-Nol were reduced by sialoadenectomy and restored in part by oral administration of EGF. We conclude that salivary glands in rats are not essential for the gastroprotection induced by De-Nol, but seem to play an important role in the ulcer healing action of this drug possibly *via* an EGF mediated mechanism.**

Recent studies in animals have shown that colloidal bismuth subcitrate (CBS; De-Nol) protects the gastric mucosa against various ulcerogens possibly due to increased mucosal production of prostaglandins (PG).[1,2]

Colloidal bismuth subcitrate is also recognised as an effective agent in the management of chronic gastroduodenal ulceration in man[3-5] and, furthermore, decreases the frequency of recurrence of ulcerations.[6] The mechanisms of these ulcer healing properties of De-Nol have not been explained, but they have been attributed to its selective binding to the ulcer base to form a protective barrier against acid-pepsin attack and to the enhancement of the re-epithelialisation of the ulcerated mucosa.[7-9] Because epidermal growth factor (EGF) is secreted by salivary glands in large quantities[10,11] and also displays gastroprotrective,[12,13] ulcer healing,[14,15] and trophic properties,[16] we reasoned that it may contribute to the action of De-Nol on the stomach.

This study was undertaken to determine the possible role of EGF in the gastroprotective and ulcer healing effects of De-Nol.

Methods

RATS

Male Wistar rats, weighing 200–250 g, were used in studies on gastroprotection, ulcer healing, and mucosal growth.

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EXPERIMENTS WITH ACUTE GASTRIC MUCOSAL LESIONS

Experiments with acute gastric lesions induced by absolute ethanol[1] were carried out in two series (series A and B) of rats. In the series A, the submandibular glands were removed bilaterally under ether anaesthesia one week earlier. In the series B, involving control rats with intact salivary glands, sham operation (longitudinal transection of the skin on the neck without removal of salivary glands) was done under similar conditions also one week before the experiment. The following experimental groups were included into the series A and B; group I with untreated stomach and without (20 rats) or with sialoadenectomy and without EGF (10 rats) or with EGF pretreatment (10 rats); group II with the stomach treated with 100% ethanol and with intact salivary glands (18 rats) or with sialoadenectomy and without EGF (20 rats) or with EGF pretreatment (20 rats); group III with the stomach treated with De-Nol alone, and intact salivary glands (10 rats) or with sialoadenectomy and without EGF (10 rats) or with EGF pretreatment (10 rats), group IV with stomach treated with the combination of De-Nol and 100% ethanol and with intact salivary glands (10 rats) or with sialoadenectomy and without EGF (10 rats) or with EGF pretreatment (10 rats); group V with stomach treated with the combination of 16,16 dm PGE_2 and 100% ethanol and with intact salivary glands (eight rats) or with sialoadenectomy and without EGF (eight rats) or with EGF (eight rats) pretreatment; group VI with stomach treated with the combination of EGF and 100% ethanol and with intact salivary glands (10 rats) or with sialoadenectomy and without EGF (10 rats) or with EGF pretreatment (10 rats).

The active ingredient of De-Nol formulation, colloidal bismuth subcitrate, was a gift from Dr D W R Hall, Department of Biological Research, Gistbrocades, Delft, The Netherlands. Colloidal bismuth subcitrate was dissolved in water and administered po in a dose of 40 mg/kg (in 1 ml volume) about 30 minutes before the start of the administration of absolute ethanol (1 ml) in rats of both series (A and B). For comparison, 16,16 dimethyl PGE_2 (gift from Dr J Pike, The Upjohn Co, Kalamazoo, Michigan, USA) or EGF (gift from Dr H Gregory, ICI, England) were used po 30 minutes before absolute ethanol in a single dose of 100 μg/kg (in 1 ml volume). These doses were found previously to prevent the haemorrhagic lesions induced by absolute ethanol or acidified aspirin in the rat stomach.[12] In addition, EGF was added for seven days to the drinking water in a dose of 30 μg/kg/day in groups of rats from the A series starting with the first day after operation (sialoadenectomy). These animals received De-Nol or $DMPGE_2$ 30 minutes before absolute ethanol as in other groups. One hour after ethanol administration the animals were killed by a blow to the head, the stomach was removed and the area of gastric lesions was measured planimetrically (Morphomat, Carl Zeiss, Berlin, Germany).

In the tests with De-Nol alone and De-Nol plus absolute ethanol, done on intact and sialoadenectomised animals with and without pretreatment with EGF, the mucosal generation of PGE_2 was determined. Immediately after killing the animals, the abdomen was opened and the stomach was clamped at the cardia and the pylorus. The stomach was washed of debris using 2 ml 0·02 M Tris buffer and then opened along the great curvature. A large (50 mg) biopsy of oxyntic mucosa was taken from the oxyntic gland area not involved in the macroscopic lesions, to determine the capability of the mucosa to generate PGE_2 as previously described.[1,12] All experiments were carried out on 24 h fasted rats.

EXPERIMENTS WITH CHRONIC GASTRIC AND DUODENAL ULCERATIONS

The following groups of rats with chronic gastric and duodenal ulcers induced by acetic acid and with the sialoadenectomy (series A) or with intact salivary glands (series B) were used; group I (30 rats) treated with De-Nol for seven or 14 days, group II treated with EGF (30 rats) for seven or 14 days, and group III control rats (30 rats) treated with vehicle only for seven or 14 days.

Chronic gastric and duodenal ulcers were produced using our modification[17] of the acetic acid method described by Okabe *et al.*[18] The animals were fasted for 24 h and under ether anaesthesia, their abdomen was opened and the stomach and duodenum exposed. A plastic mould of 4·2 mm diameter was applied tightly to the serosal surface of the anterior wall of the stomach just proximal to the antral gland area and then to the serosal surface of the duodenum about 5 mm beyond the pylorus. About 70 μl 100% acetic acid was poured through the mould on to the surface of the stomach and allowed to remain there for 20 s. Similarly, 70 μl 75% acetic acid was applied to the duodenum for 10 s. This method was found to cause an immediate necrosis of the entire thickness of the mucosa and submucosa directly under the area of the application of acetic acid. Gastric and duodenal ulcers were formed having an initial area of about 13·8 mm^2 as described before.[17] Unlike the original technique of ulcerations described by Okabe *et al*[18] our modification resulted in the formation of ulcers which did not penetrate into the surrounding organs and which healed completely within about two to three weeks. After application of acetic acid, the animals were allowed

to recover from the anaesthesia and received only water on the day of operation (day 0). Then they were divided into various experimental groups and fed normal chow and water *at libitum*.

In rats with the submandibular glands removed or in sham operated animals acetic ulcers were induced and then the effects of De-Nol and EGF on the healing of these ulcers were tested during the period of seven to 14 days. Epidermal growth factor was added to drinking water in a dose of about 30 μg/kg/day, while De-Nol was added to drinking water at a dose of about 100 mg/kg/day. The volume of drinking water was measured each day in each experimental groups and it averaged about 30 ml/day in rats with intact salivary glands and about 45 ml/day in sialoadenectomised animals. Control groups of rats with intact or excised salivary glands and acetic acid induced gastric and duodenal ulcers, received water without De-Nol or EGF. Apart from the day of operation where the rats received only water, all animals were maintained on purina chow diet throughout the period after induction of acetic acid ulcerations.

To evaluate the effects of De-Nol and EGF on ulcer healing the animals were killed after two, seven, or 14 days after ulcer induction. Under deep ether anaesthesia the stomach and the duodenum were removed and opened for the measurement of ulcer area by planimetry.

STUDIES ON EGF RECEPTOR BINDING AND LOCATION OF LABELLED EGF IN THE GASTRODUODENAL MUCOSA

The effects of De-Nol on the binding of 125I-labelled EGF to its receptors were determined using a mouse liver particulate fraction which is a rich source of specific EGF binding sites, as documented before.[19] The livers of male mice were homogenised in 10 vol of ice cold 50 mM-Tris HCl (pH 7·4) containing 10 mM-MgCl×6 H O. The homogenate was centrifuged at 12000 *g* for 20 min and the precipitate was resuspended in an equal volume of Tris buffer containing 0·1% bovine serum albumin (Sigma Co, St Louis, MO). 125I-labelled EGF (specific activity 100 μCi/μg of total EGF, Amersham, UK) was incubated at pH 7·4 with tissue homogenate. Assays were conducted at 22°C for 60 min. The final concentration of 125I-EGF in each tube was $1{\cdot}2\times10^{-6}$ M. Displacement curves were generated by simultaneous incubation with serial dilutions of unlabelled EGF (gift from Dr H Gregory, ICI, UK), De-Nol, vehicle, or the combination of EGF and De-Nol. All experiments were carried out in duplicate and non-specific binding was subtracted before analysis of the data. Non-specific binding always accounted for less than 20% of the total binding.

The adsorption of EGF to De-Nol was examined *in vitro* by adding De-Nol (1 mg) or vehicle (control) to 500 μl of 125I-EGF dissolved in phosphate buffer adjusted to various pHs ranging from 1·5 to 8·0, and incubating the mixture for 30 min at room temperature. The mixture was then centrifuged for 10 min and EGF bound to precipitated De-Nol was harvested and measured.

In a separate series of experiments on 18 h fasted rats with chronic gastric and duodenal ulcers with intact and excised submandibulary glands, 125I-EGF (0·25 pmol) dissolved in 1 ml saline with or without De-Nol (20 g/l) was administered intragastrically by orogastric tube. The rats were then killed at 60, 120, and 180 min after EGF administration. The stomach and duodenum were removed, cut open and spread out. The ulcer area including the ulcer base with about 2 mm mucosa at the ulcer edge was excised. Similar areas of intact gastric or duodenal mucosa were removed from sites adjacent to the gastric and duodenal ulcers. The radioactivity of each sample was measured.

In another series of rats with intact or removed submandibulary glands and with gastric and duodenal ulcerations, the quantity of endogenous EGF bound to the ulcer area and intact mucosa were determined. For this purpose, the ulcer area and similar areas of intact adjacent gastric and duodenal mucosa were excised, homogenised in ice cold 0·02 M Tris HCl buffer (pH 9·6) and centrifuged, the supernatant being frozen at −20°C until EGF radioimmunoassay. The EGF antiserum raised in rabbits against EGF was used in a final dilution of 1:210000. The iodinated peptide and calibration standards were EGF (Amersham, UK). The calibration curve was carried out in charcoal stripped serum from untreated rats. The detection limit of the assay was 0·05 nmol/l and precision was about 15%.

STATISTICAL ANALYSIS

Results are expressed as means (SE). The significance of the differences between means was evaluated using Student's *t* test for unpaired values. Differences were considered significant if p was less than 0·05.

Results

GASTROPROTECTIVE EFFECTS OF DE-NOL AND 16,16 DIMETHYL PGE_2 ($DMPGE_2$) IN RATS WITH INTACT AND EXCISED SUBMANDIBULARY GLANDS

Severe haemorrhagic lesions were found in the stomachs exposed to 100% ethanol in rats with intact and resected submandibulary glands. The mean lesion area in rats with intact glands averaged 78 (12) mm^2 and that in sialoadenectomised rats was 52 (14)

Table 1 *Effects of De-Nol (40 mg/kg), 16,16 dimethyl PGE_2 (10 μg/kg) and EGF (100 μg/kg) administered po on the area (mm^2) of gastric lesions induced by 100% ethanol in rats with intact salivary glands (series B) and sialoadenectomised rats (series A) without and with pretreatment with EGF (30 μg/kg/day)*

Group	*Intact salivary glands*		*Sialoadenectomy*			
			Without EGF		*With EGF*	
	Ulcer area (mm^2)	*PG gener. (ng/g)*	*Ulcer area (mm^2)*	*PG gener. (ng/g)*	*Ulcer area (mm^2)*	*PG gener. (ng/g)*
Intact stomach (untreated)	0	470 (61)	0	310 (50)	0	510 (68)
100% ethanol (control)	78 (12)	620 (70)	52 (14)	436 (50)	67 (16)	642 (56)
De-Nol alone	0	578 (52)	0	530 (63)	0	566 (49)
De-Nol + 100% ethanol	3* (1)	680 (74)	4* (3)	492 (60)	6* (4)	710 (84)
16,16 $dmPGE_2$ + 100% ethanol	4* (1)	NT	8* (3)	NT	3* (1)	NT
EGF + 100% ethanol	25* (8)	650 (49)	31* (9)	540 (68)	28* (5)	675 (72)

PG gener: prostaglandin generation (ng/g of wet tissue weight); NT: not tested. Mean (SE) of eight to 20 tests on eight to 20 rats.
* indicates difference from the control values obtained with 100% ethanol alone.

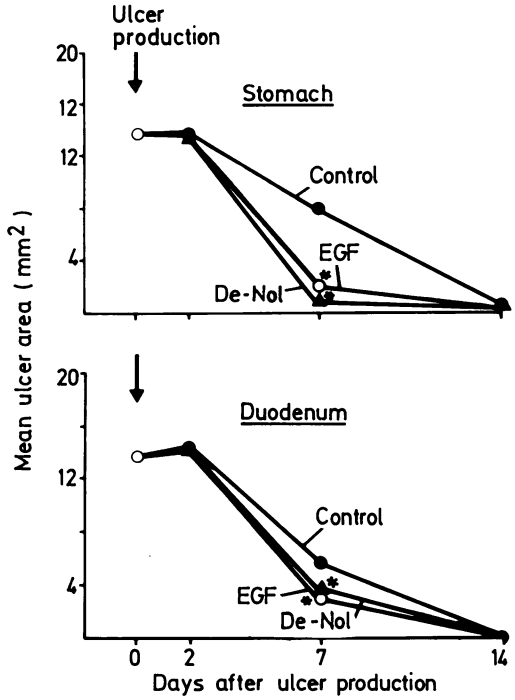

Fig. 1 *Mean (SE) area of chronic gastric and duodenal ulcerations induced in rats after serosal application of acetic acid. Measurements were at two, seven, and 14 days after the ulcer induction in rats of series B with intact salivary glands, with or without oral administration of De-Nol (100 mg/kg/day) or EGF (30 μg/kg/day). Zero measurement shows the area of application of acetic acid the initial area of damage. * Indicates statistically significant decrease in ulcer area as compared with control value.*

mm^2. The difference in the mean area was not statistically significant. De-Nol (40 mg/kg) and $dmPGE_2$ (10 μg/kg) administered po in a single dose to intact rats 30 min before ethanol almost completely protected the mucosa against the damaging effects of ethanol (Table 1). After sialoadenectomy, De-Nol resulted in similar reduction in ulcer area as in rats with intact submandibular glands. Also $dmPGE_2$ reduced the ethanol induced gastric lesions to the same extent as in rats with intact glands. In sialoadenectomised rats pretreated with oral EGF for seven days, De-Nol and $dmPGE_2$ produced the protection against ethanol injury similar to that observed in rats with intact salivary glands. A single dose of EGF given po before ethanol was only partly effective against ethanol injury both in intact and sialoadenectomised rats (Table 1).

In tests on rats with intact salivary glands the mucosal generation of PGE_2 after the administration of De-Nol alone or De-Nol plus 100% ethanol were significantly higher than those recorded in the untreated stomach (Table 1). After sialoadenectomy, the untreated gastric mucosa generated somewhat smaller amounts of PGE_2 than in rats with intact salivary glands but the difference was not statistically significant. De-Nol alone and combined with 100% ethanol resulted in similar increment in the bio-synthesis of PGE_2 than in rats with intact salivary glands. In sialoadenectomised rats pretreated with EGF mucosal generation of PGE_2 in intact mucosa and that treated with De-Nol and/or ethanol did not differ from that in rats with intact salivary glands.

EFFECTS OF DE-NOL AND EGF ON HEALING OF CHRONIC GASTRIC AND DUODENAL ULCERS

In control rats with intact and removed salivary glands, serosal application of acetic acid resulted in the formation of gastric and duodenal ulcers in all rats treated (Fig. 1). The initial area of ulcer production was 13·8 mm^2 and that, measured at two days after ulcer induction, was 13·2 (1·4) mm^2 in the stomach and 14·5 (1·8) mm^2 in the duodenum. After seven days, the ulcers were found in all control rats but there was a significant decrease in the ulcer size to respective values of 8·0 (1·6) and 7·4 (1·0) mm^2. Two weeks after ulcer induction, the area of gastric ulcers was reduced to about 0·8 (0·2) mm^2 and in six of eight

Table 2 *Effects of De-Nol, EGF and their combination on area of gastric and duodenal ulcerations induced by serosal application of acetic acid in rats of series A with resected submandibular glands. Control group includes sialoadenectomised rats (series A) with gastric and duodenal ulcers without De-Nol or EGF treatment*

	Mean ulcer area (mm^2)					
	Gastric ulcers			Duodenal ulcers		
Group	2 day	7 day	14 day	2 day	7 day	14 day
Control	14·3 (2·1)	10·4 (1·7)	3·1 (0·8)	15·1 (2·0)	6·2 (1·4)	0·6 (0·2)
De-Nol alone (100 mg/kg/day)	14·4 (1·6)	6·2* (1·0)	1·9* (0·6)	15·0 (1·8)	4·1* (0·8)	0·2 (0·1)
EGF alone (30 µg/kg/day)	14·0 (1·9)	7·8 (2·1)	2·0* (0·7)	14·9 (2·1)	4·8* (1·1)	0·4 (0·2)
EGF (30 µg/kg/day) +De-Nol (100 mg/kg/day)	14·1 (2·4)	4·2* (1·6)	1·2* (0·2)	15·6 (2·2)	3·2* (1·4)	0·6 (0·2)

Mean (SE) of 10–20 rats. * indicates statistically significant decreases below the control values.

rats the ulcers were healed completely. The duodenal ulcers healed in all animals. In rats treated with De-Nol, there was a significant decrease in the ulcer area seven days after ulcer induction in the stomach and duodenum; the ulcers were recorded in the stomach in three of 10 rats and in the duodenum in five of 10 animals, respectively. Similarly, EGF treatment resulted in a significant reduction in the ulcer area and the ulcer incidence both in the stomach and the duodenum.

In sialoadenectomised rats the area and the incidence of ulcers seven and 14 days after their induction, were significantly larger in the stomach but not in the duodenum than those in rats with intact salivary glands (Table 2). De-Nol or EGF given alone to sialoadenectomised rats resulted in the significant reduction in mean ulcer area. When combined with EGF, De-Nol in sialoadenectomised rats decreased the area of ulcers to a greater extent than when given alone but this difference in the ulcer area was not statistically significant (Table 2).

EFFECTS OF DE-NOL ON BINDING OF EGF TO THE EGF RECEPTORS AND TO THE ULCERATED MUCOSA

De-Nol in a concentration of 20 g/l did not affect the binding of 125I-EGF to its liver binding sites in the presence of concentrations of unlabelled EGF between 5 and 25 nmol/l. De-Nol alone in various concentrations (5–25 g/l) without unlabelled EGF did not influence the binding of EGF to its receptors and this binding averaged about 98% of the total amount bound in the absence of De-Nol (Fig. 2).

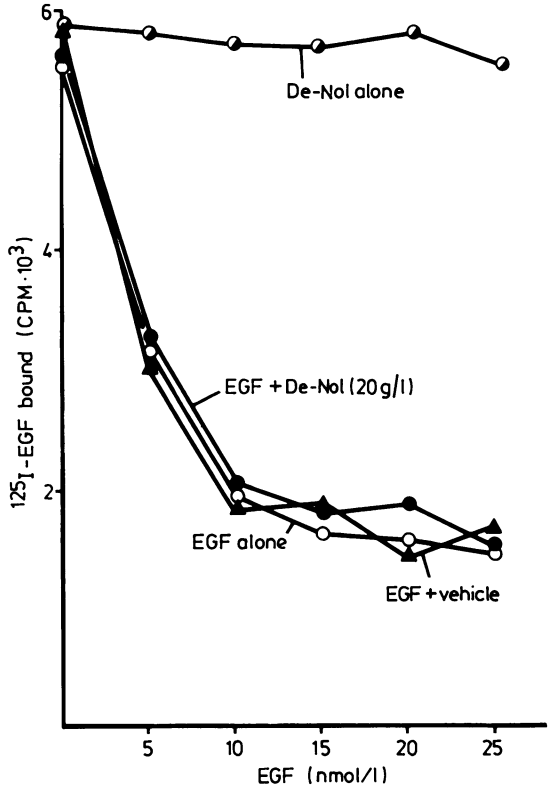

Fig. 2 *Influence of the De-Nol alone in concentrations ranging from 5–25 g/l (upper line) and De-Nol (20 g/l) or vehicle in the presence of various concentrations (5–25 nmol/l) of 'cold' EGF on binding of labelled 125I-EGF to its receptors in the mouse liver. Mean of six tests on six separate liver preparations.*

Labelled EGF added *in vitro* to the solutions of De-Nol adjusted to various pH ranging from 1·5 to 8·0 was found to be coprecipitated with CBS in gradually increasing amounts as the pH of solution was reduced below pH 6·0. Significant binding of EGF to De-Nol occurred at pH 4·5 and it reached about 77% at pH 1·5 (Fig. 3).

The distribution of the 125I-labelled EGF (measured at 180 min after introduction into the rat stomach) between the ulcer area and adjacent intact mucosa is shown in Figure 4. There was a measurable amount of EGF bound to the intact mucosa both in the stomach and duodenum. The ulcer area in the stomach (but not in the duodenum) accumulated about twice as much labelled EGF as the intact mucosa. In rats treated with De-Nol (100 mg/kg) there was further increase in the accumulation of labelled EGF in the area of gastric (but not duodenal) ulcers observed at 60, 120, and 180 min after

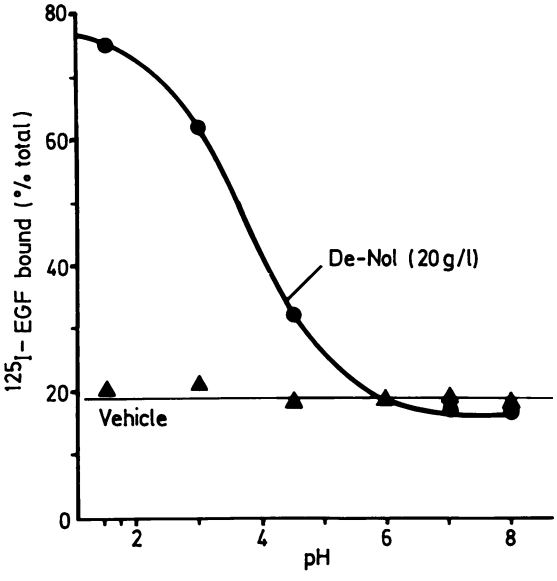

Fig. 3 *Coprecipitation of labelled EGF by De-Nol (20 g/l) or vehicle solutions adjusted to pHs ranging from 1·5 to 8·0. Results are expressed in per cent of total labelled EGF added to the solutions.*

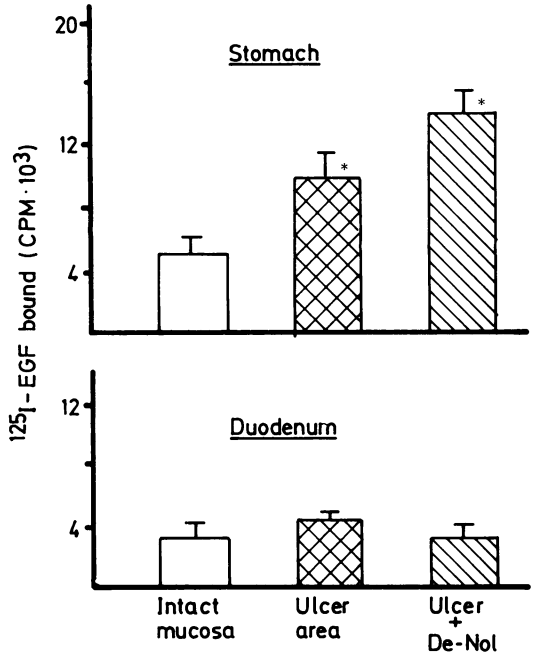

Fig. 4 *Location of labelled EGF at 180 min after introduction in gastric or duodenal ulcerations and the adjacent intact mucosa in rats with and without administration of De-Nol (100 mg/kg). Mean (SE) of six tests on six rats. * indicates statistically significantly ($p<0·05$) greater EGF than intact mucosa.*

administration of labelled EGF. No significant changes in the presence of labelled EGF in the intact mucosa between rats treated and untreated with De-Nol were observed and these data have not been included.

The distribution of endogenous immunoreactive EGF in the intact and ulcerated gastric and duodenal mucosa in rats with or without De-Nol treatment is shown in Figure 5. The amounts of EGF in the area of gastric and duodenal ulcers were significantly higher than in the intact adjacent mucosa and this difference was further increased 180 minutes after pretreatment with De-Nol. The amounts of EGF in the duodenum within the ulcer area were significantly larger than in the stomach, in De-Nol treated and untreated rats. In the adjacent duodenal mucosa the amounts of EGF tended to be larger than in gastric mucosa but the difference was not statistically significant.

Discussion

This study provides evidence that (De-Nol) protects the gastric mucosa against the damage caused by absolute ethanol and enhances the healing rate of chronic gastric and duodenal ulcers, and that the ulcer healing effects of De-Nol may be mediated, at least in part, by epidermal growth factor (EGF).

Previous studies have shown that colloidal bismuth subcitrate exhibits gastroprotective action against various ulcerogens including absolute ethanol and acidified aspirin.[12] Because De-Nol was found to stimulate the mucosal generation and luminal release of cytoprotective prostaglandins (PG) it has been suggested that this protection is mediated by PG. The finding that De-Nol also protects against the injury caused by aspirin, which is a potent inhibitor of PG cycloocygenase,[12] indicates that this drug may activate some other as yet unknown protective mechanisms. This study shows that EGF, which is produced by salivary[10] and Brunner's glands,[11] produces partial protection of the gastric mucosa against ethanol injury when administered orally at a dose that exceeds the amount of the peptide produced endogenously.[19,20] This protection probably represents a pharmacological effect of EGF because the removal of the submandibular glands, and thus the major endogenous source of EGF, does not appear to interfere with gastroprotection afforded by De-Nol. Also the gastroprotection elicited by $dmPGE_2$ was not altered by sialoadenectomy. These results seem to indicate that EGF is not essential for indirect (De-Nol) or direct ($dmPGE_2$) gastroprotection. This is in keeping with previous observation that EGF by

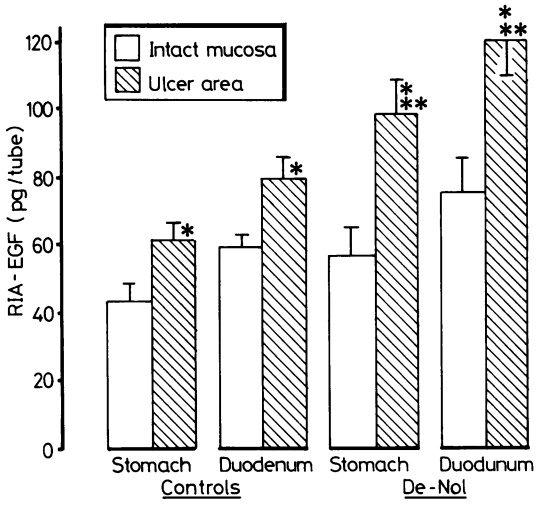

Fig. 5 *Content of immunoreactive EGF in the ulcer area and the adjacent mucosa of the stomach and duodenum in rats with and without pretreatment with De-Nol (100 mg/kg/day). Mean (SE) of 10–12 rats. ** indicate statistically significantly ($p<0.05$) greater than untreated controls.*

itself does not stimulate the mucosal generation of PG,[12] although this does not exclude the possibility that the presence of EGF and normal mucosal growth and integrity are necessary conditions for adequate responsiveness of the mucosa to various agents.[21] Such a notion would remain in agreement with the finding that sialoadenectomy increases the susceptibility of the mucosa to the damage by bile salts,[22] possibly because of decrease in tissue growth and mucosal integrity, and that pentagastrin, which is a known trophic factor, reverses the susceptibility of the stomach to stress ulceration.[23]

The major finding of this study is that colloidal bismuth enhances the healing rate or chronic gastric and duodenal ulcerations induced in rats by serosal application of acetic acid. Although De-Nol was reported to heal chronic gastroduodenal ulcerations in man[3-6] our study provides the first experimental evidence that De-Nol is effective in a fully standardised experimental model of chronic ulceration. The question remains as to the possible mechanism of the favourable influence of De-Nol on ulcer healing. As exogenous EGF in the same ulcer model also increased the ulcer healing[14,15] we reasoned that this peptide may contribute to the ulcer healing of De-Nol. The possibility that De-Nol may speed the ulcer healing by affecting the binding of EGF to its receptor sites in the gastric mucosa has not been examined before. Our *in vitro* studies showed that neither De-Nol alone nor its combination with EGF affects the binding of labelled EGF to its receptors. Epidermal growth factor was found to accumulate, however, in the ulcer area to a greater extent than in the intact mucosa and this has been documented using both radiolabelled EGF introduced into the stomach and direct measurement by radioimmunoassay of the tissue content of endogenous EGF. Ulcerated mucosa in the stomach can accumulate larger amounts of EGF than the adjacent intact mucosa. The only difference between these two experiments was that the amounts of EGF measured by radioimmunoassay were relatively larger in the duodenum than in the stomach, whereas those determined by binding of labelled EGF were higher in the stomach. This difference could be explained by binding of endogenous EGF released locally to its receptors in the duodenum so that they were not available for exogenous labelled EGF. The opposite was true for the stomach, where salivary EGF present in smaller concentrations because of its dilution by the gastric contents, did not interfere with the binding of labelled EGF to its mucosal receptor sites.

De-Nol is also capable of coprecipitating with luminal EGF in a pH dependent manner so that most of the endogenously released peptide in the gastric lumen is bound to De-Nol. This binding of EGF by De-Nol seems to be an important factor because the drug also selectively chelates with proteinaceous material of the ulcerated mucosa to form a dense coating on the base of an ulcer or mucosal defect.[7,8] This probably explains why labelled EGF accumulated in much larger amounts within the ulcer area when given together with De-Nol than without this drug. De-Nol was also effective in accumulating EGF in the area of duodenal ulcers but this could not be detected by simple measurement of the radioactivity of labelled exogenous EGF administered into the stomach but required the use of radioimmunoassay, probably because the EGF binding sites were already saturated by endogenous peptide produced locally in Brunner's glands.[11] The removal of free EGF from the solution by De-Nol does not appear to interfere with the ability of this peptide to bind to its hepatocyte receptors because the receptor binding assay was carried out at pH above that at which EGF coprecipitate with De-Nol.

Our finding that the ulcerated mucosa binds larger amounts of EGF than the adjacent intact mucosa could be explained by the increased number of EGF receptors in the cells in the area of rapid re-epithelialisation and tissue repair. The EGF receptors have been identified in epithelial cells as well as in fibroblasts[24-26] of the gut. The augmentation by De-Nol of the accumulation of EGF in the ulcer area makes the peptide available locally for ulcer

healing in a larger concentration and for a longer time than when EGF alone is present. This effect could be attributed to the physical adsorption of EGF to De-Nol or to the binding of such EGF-loaded drug with the proteins of the ulcerated mucosa. Because exogenous EGF given into the stomach[27] or the intestines[28] retains its trophic effects on the gastroduodenal mucosa, it is reasonable to assume that endogenous EGF accumulated in the ulcer area also has growth promoting action that accelerates ulcer healing similar to that of gastrin.[29] This is supported by our finding showing that sialoadenectomy delays ulcer healing and that exogenous EGF reverses such a retardation of ulcer healing resulting from the removal of the endogenous source of EGF. De-Nol appears to enhance this action of EGF by binding the peptide and accumulating it on the ulcerated mucosa. This mechanism might play some role in the healing of chronic peptic ulcers in man by De-Nol.

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