Virchows Arch (1998) 432:371–375

ORIGINAL ARTICLE

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Gastrin stimulates epithelial cell proliferation in the oesophagus of rats

Received: 15 September 1997 / Accepted: 23 October 1997

Abstract Gastrin can induce mitotic stimulation in the oxyntic mucosa of the stomach, sometimes leading to abnormal growth. We examined whether gastrin was able to influence cell proliferation in the oesophageal epithelium. Rats were treated with gastrin, omeprazole or saline for 3 days, or were subjected to fundectomy or sham operation. Bromodeoxyuridine labelling (LI) and mitotic (MI) indices were counted in the proliferative zone of the squamous epithelium. Infusion of exogenous gastrin, treatment with omeprazole or fundectomy raised the LI and the MI values in the oesophageal epithelium, indicating that gastrin stimulates cell proliferation in the oesophageal mucosa in the rat.

Key words Gastrin · Oesophagus · Cell kinetics · Rats

Introduction

The hypothesis that gastrin acts as a trophic hormone throughout the entire digestive tract [4, 5, 16, 17, 21, 25] is controversial [7, 10]. A trophic effect of physiological concentrations of gastrin has not been confirmed in gastric antral mucosa [3], pancreas [10], small intestine [7, 24], colon [8] or gallbladder [19]. In fact, the only part of the gut that is unanimously recognised as a target for the growth-promoting effect of gastrin is the oxyntic mucosa of the stomach [13, 29]. In vivo mitotic activity of both epithelial stem cells and enterochromaffin-like (ECL) cells in this tissue is selectively stimulated by gastrin in experimental animals [12, 28].

The trophic effects of gastrin became a matter of concern when potent inhibitors of acid secretion, which significantly increase the serum gastrin concentrations, were made available. Theoretically, mitogenesis might allow an increased frequency of mutation. Effects on serum gastrin levels raised concerns, especially in the chronic treatment of ulcer patients. However, the clinical significance of ECL-cell hyperplasia, ECL-cell tumours or gastric polyps observed after long-term use of proton pump inhibitors does not outweigh the benefit of these drugs in the modern treatment of peptic disease. The safety of chronic use of acid-blocking drugs in the treatment of gastro-oesophageal reflux disease may also be questioned. Chronic administration of a proton pump inhibitor is increasingly used in the treatment of patients with reflux oesophagitis, but the effect of gastrin on epithelial growth in the oesophagus has not been explored.

In the present study, we examined the effect of both exogenous and endogenous gastrin on proliferative activity in the oesophageal epithelium of rats.

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Materials and methods

Male Sprague-Dawley rats weighing 250–300 g were used. Unless otherwise specified, the animals had free access to tap water and standard rat food pellets. The experiments and operative procedures were carried out in accordance with the principles of laboratory animal care, the rules laid down by the Ethical Committee on Animal Research of the Free University of Brussels, the Belgian legislation and the European Economic Community regulations.

In a first experiment, 18 rats were anaesthesised with an i.p. injection of 300 mg/kg of chloral hydrate. Through a small longitudinal neck incision, the right jugular vein was dissected free and a catheter was inserted and tunnelled subcutaneously to the back of the neck. Then it was connected to an external metal tether and a liquid swivel joint system, allowing the animals to move freely. The animals were randomly divided into three groups of 6 animals each. Using a syringe pump (World Precision Instruments, Aton, UK) each animal was given a continuous infusion of 10 ml/24 h of 1% bovine serum albumin (Sigma, St. Louis, Mo.) in saline. Drug administration was started on the day after the operation. In the first group rat gastrin-17 (Sigma) was added to the infusion medium, delivering a dose of 2.5 nmol/kg per h (=5.32 μg/kg per h) of gastrin in 3 days. In the second group 20 μmol/kg (=6.91 mg/kg) of the i.v. solution of omeprazole was injected once daily at 9 a.m. through the catheter, also for 3 days. In the third group only the vehicle solution was given. Thereafter, the rats were killed by exsanguination, 1 h after an i.p. injection of 60 mg/kg of bromode-

Table 1 Serum gastrin concentrations and proliferative parameters in the progenitor zone of the oxyntic mucosa of vehicle treated control rats (n=6) and animals treated with a continuous infusion of rat gastrin-17 (2.5 nmol/kg per h; n=6) or a daily bolus of omeprazole (20 µmol/kg per day; n=6)

	Serum gastrin concentrations (pg/ml)	Oxyntic mucosa	
		Labelling index (%)	Mitotic index (%)
Control	59.14 ± 15.18	3.43 ± 0.19	0.11 ± 0.02
Omeprazole	315.32 ± 88.28*	7.50 ± 0.61 **	0.26 ± 0.04 **
Gastrin	319.52 ± 72.87**	9.70 ± 1.77 **	0.35 ± 0.05 **

* $P < 0.05$; ** $P < 0.01$

oxyuridine (BrdU; Sigma). The oesophagus and the oxyntic area of the stomach were excised and fixed for 24 h in 10% neutral formalin at 4°C. Blood was collected and centrifuged and serum was kept frozen at –80°C for serum gastrin assay.

In a second experiment 45 animals were randomly subdivided into three groups of 15 rats each; these were kept fasted for 24 h before surgery. Chloral hydrate anaesthesia was used. In the first group, fundectomy was carried out through a median laparotomy by resecting the oxyntic gland area of the stomach and suturing the rumen to the antrum, using interrupted sertix silk (5/0) sutures. A Heineke-Miculicz pyloroplasty was carried out to anticipate pylorospasm resulting from vagal damage. In the second group a simple transection through the gastric corpus was made without any resection, followed by reanastomosis and pyloroplasty. The animals of the third group were not submitted to anaesthesia or surgery.

After a 2-week recovery period, all the animals were kept fasted for 24 h and were killed 1 h after one i.p. injection of BrdU. The oesophagus was resected and fixed in 10% neutral formalin and blood was taken for serum gastrin assay as in the first experiment.

Mucosal samples were cut perpendicular to the mucosal surface and were stained for BrdU uptake. For this purpose an anti-BrdU antibody (Dako, Glosbrup, Denmark) and the immunoperoxidase technique were used, following a technique described elsewhere [18]. The uptake of BrdU in the cells was indicated by the selective brown staining of the cell nucleus.

In a preliminary study in 10 normal rats, the limits of the normal progenitor zone of the oesophageal epithelium and that of the gastric oxyntic glands were determined by establishing a spatial distribution histogram of the labelled cells for each. For this purpose, the positions of 1000 labelled cells in each tissue specimen were noted and expressed as the number of cells above the basal cell layer in the oesophageal epithelium or above the uppermost chief cell in each gastric oxyntic gland examined. Since more than 95% of the labelled cells were observed within the two basal cell layers of the oesophageal epithelium, both labelling (LI) and mitotic (MI) index counts were restricted to this area, which is called the proliferative zone [6]. In the oxyntic glands, the progenitor zone was defined as the area between the uppermost chief cell and the uppermost parietal cell in each gland [28].

The percentages of labelled cells, or LI, and of mitotic figures, or MI, were determined in a total of 2000 nucleated epithelial cells in the progenitor zone of the oesophagus and in the progenitor zone of 20 well-defined glands of the gastric oxyntic gland area in each animal.

For the gastrin assays, a double-antibody liquid-phase ^{125}I-radioimmunoassay (Diagnostic Products Corporation, Los Angeles, Calif.) was used as described in detail elsewhere [11, 26]. Serum concentrations were expressed as picogram equivalents of synthetic human gastrin-17 per millilitre.

Results were expressed as means ± SEM, Student's t-test for unpaired samples was used after arc sine transformation of the proportions. The significance of correlation was calculated using Fisher's r-to-z transformation. Statistical significance was set at $P < 0.05$.

Results

Serum gastrin values were increased 5-fold both in the animals receiving exogenous gastrin ($P < 0.01$) and in those given omeprazole ($P < 0.05$) compared with control rats (Table 1).

Both LI and MI values were increased in the oxyntic glands in response to gastrin ($P < 0.01$) or to omeprazole ($P < 0.01$) (Table 1). In the oesophageal epithelium both proliferative parameters were increased both in the gastrin treated ($P < 0.01$) and in the omeprazole-treated ($P < 0.01$) groups compared with control rats (Fig. 1). There was a significant correlation between the serum gastrin concentration and the LI ($n = 18$, $r = 0.59$, $P < 0.05$) on the one hand and between the serum gastrin

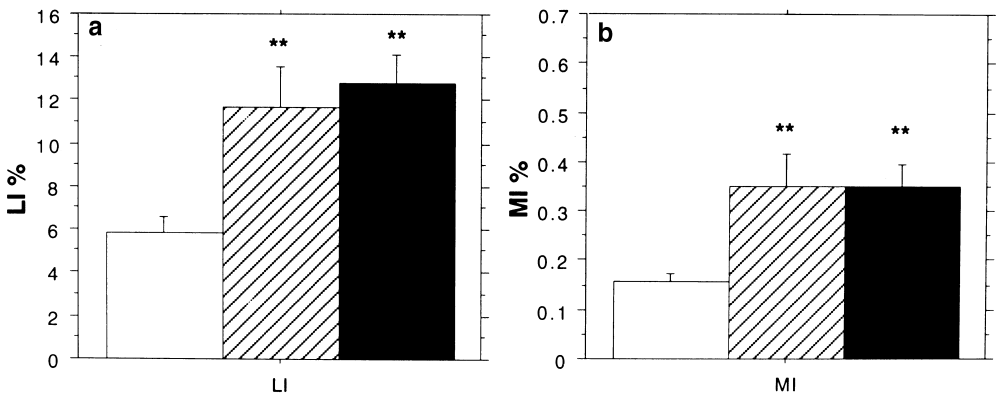

Fig. 1a Labelling index (LI %) in the oesophageal epithelium of controls (□) and of rats treated with gastrin (■) or omeprazole (▨). ** $P < 0.01$. **b** Mitotic index (MI%) in the oesophageal epithelium of controls (□) and of rats treated with gastrin (■) or omeprazole (▨) ** $P < 0.01$

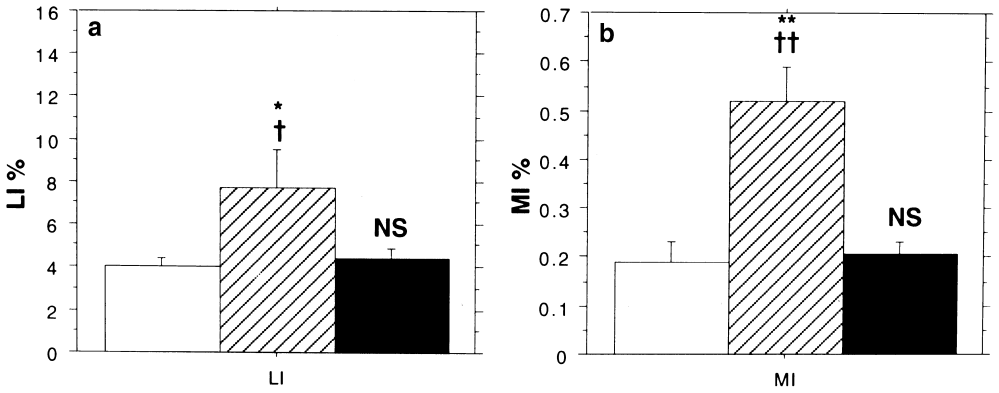

Fig. 2a LI in the oesophageal epithelium of control rats (□) and of rats after fundectomy (▨) or simple gastric transection (■). NS not significant; * $P < 0.05$, vs controls; † $P < 0.05$, vs sham operated animals. Mitotic index in the oesophageal epithelium of control rats (□) **b** or rats after fundectomy (▨) or simple gastric transection (■) NS not significant, ** $p < 0.01$, vs controls; †† $P < 0.01$, vs sham operated animals

Table 2 Serum gastrin concentration in intact control rats (n=15), fundectomised rats (n=12) and operated control rats, subjected to simple gastric transsection (n=15)

Operation	Serum gastrin concentration (pg/ml)
Control	53.74 ± 3.27
Fundectomy	358.18 ± 44.33**††
Transection	62.51 ± 4.88 NS

NS not significant; ** $P < 0.01$ vs controls; †† $P < 0.01$ vs operated control

concentration and the MI (n = 18, r = 0.52, $P < 0.05$) on the other.

After fundectomy 12 animals were available for the study, since 2 animals died postoperatively and 1 animal was excluded because of a pyloric stenosis and gastric dilatation. Serum gastrin levels increased more than 5-fold ($P < 0.01$) compared with the controls. A simple gastric transection did not affect the serum gastrin concentrations (Table 2).

LI ($P < 0.01$) and MI ($P < 0.01$) were higher in the fundectomy group than in the normal controls or in the transected group (Fig. 2). The proliferative parameters in the controls and in the transected rats were similar.

There was a significant correlation between the serum gastrin level and the LI (n = 43, r = 0.34, $P < 0.01$) and between the serum gastrin level and the MI (n = 43, r = 0.59, $P < 0.01$).

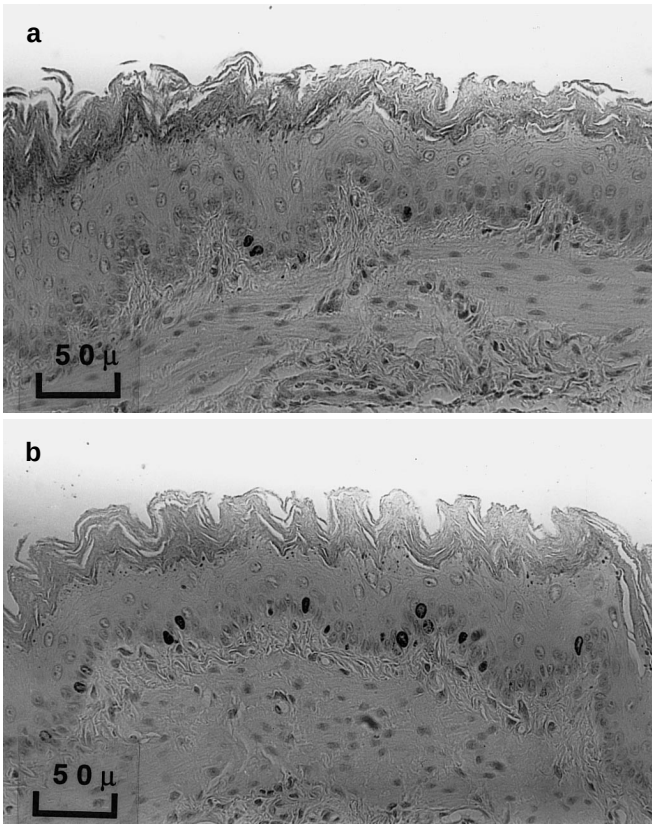

Fig. 3 Bromodeoxyuridine-labelled cells in the progenitor-zone of the oesophageal epithelium **a** of an intact control rat and **b** of a rat after a 3-day intravenous treatment with gastrin-17 (2.5 nmol/kg per h) 400×

Discussion

The possibility that gastrin might stimulate mitotic activity in the oesophagus has never been investigated. Recent studies have reported an increased incidence of oesophageal adenocarcinoma [2, 14] in Western countries, and the link with gastro-oesophageal reflux disease seems to be well established [9, 15, 27, 30]. Since practically all patients suffering from severe reflux oesophagitis are treated with potent acid-blocking drugs, investigations of a possible mitogenic effect of gastrin in this organ could be interesting. In the present study we examined whether elevated serum gastrin concentrations influenced mitotic activity in the oesophageal epithelial cells in rats.

Supramaximal doses of gastrin or of pentagastrin have been used in most previous studies on the trophic effect of gastrin in the gut, and the physiological significance of the effects observed has therefore been much debated. In the present study, infusion of a relatively low dose, namely 2.5 nmol/kg per h of rat gastrin-17 was used; this should increase serum gastrin levels in rats by 160–500%. Using these doses previously, we had ob-

served that it was possible to mimic endogenous gastrin levels resulting from achlorhydria [23]. However, omeprazole at a dose of 20 μmol/kg per day is known to abolish acid secretion in the rat [20]. In the present study, infusion through venous catheterisation was preferred to gastric intubation because mechanical irritation of the oesophagus by the tube could have influenced local cell renewal. The surgical resection of the acid-secreting part of the mucosa also induced endogenous hypergastrinaemia while avoiding the administration of an acid-blocking drug. The estimation of LI and MI values showed that administration of gastrin or of omeprazole stimulated the epithelial cell proliferation in the oesophagus (Fig. 3). Concomitant stimulation of cell proliferation in the gastric oxyntic glands confirmed earlier findings [23].

Previous studies on cell renewal in the canine oesophagus had indicated that gastric acid alone is able to stimulate oesophageal epithelial mitotic activity [6]. Increased oesophageal reflux of acid under the effect of gastrin-induced acid hypersecretion could therefore be held responsible for the observed mitotic response after gastrin infusion. However the simultaneous observation of a stimulatory effect of an acid-inhibitory drug was at odds with this latter explanation. A direct effect of the omeprazole molecule on epithelial cell replication was unlikely: elimination of acid secretion by fundectomy also induced a significant proliferative stimulation in the oesophagus. Previous studies on the mitotic stimulation of ECL cells in omeprazole-treated rats have indicated that omeprazole per se was not responsible [28]. Elevated serum gastrin concentrations were the common denominator in the animals subjected to fundectomy, those given omeprazole and those receiving synthetic gastrin. Therefore, knowing the stimulatory effect of gastrin on cell proliferation in the stomach, we felt it was reasonable to consider that gastrin was the main factor responsible for the observed response. A significant, albeit weak, correlation between the serum gastrin concentrations and the two proliferative parameters also favoured a causal relationship between serum gastrin and increased cell proliferation in the oesophagus.

The possibility that a sustained stimulation of epithelial cell proliferation in this organ could eventually lead to the onset of abnormal growth patterns was an attractive working hypothesis. The opportunity to introduce an additional pharmacological stimulant of cell replication to the local effect of the gastroduodenal refluxate [1, 22] should perhaps be taken into consideration when chronic drug administration over a long period is required. However, the recently observed increase in incidence of human oesophageal cancer relates to adenocarcinoma and not to squamous cell carcinoma. Therefore, a link between mitotic stimulation in the squamous epithelium, glandular metaplasia in this epithelium and, eventually, adenocarcinoma, is far from being established.

Acknowledgements This work was supported in part by the Nationaal Fonds voor Wetenschappelijk Onderzoek van België.

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