Effect of gastrin on liver regeneration after partial hepatectomy in rats

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Abstract

Gastrin has been shown to be an important trophic hormone for the mucosa of the stomach and the proximal intestine. In the present study the effect of gastrin on liver regeneration after partial hepatectomy in rats was investigated. After partial hepatectomy a significant rise in the concentration of gastrin in portal venous blood was found six, 12, and 18 hours after 70% hepatectomy. The effect of changes in the endogenous gastrin concentration on the liver regeneration was investigated in rats subjected to antrectomy or to fundectomy. Partial hepatectomy was done three weeks after the primary surgery. We found antrectomy to decrease liver regeneration, whereas fundectomy had no effect. Administration of pentagastrin 300 µg/kg sc three times daily for two and four days after partial hepatectomy significantly increased the rate of liver regeneration compared with controls. This study suggests that gastrin has a hepatotrophic effect. Whether this effect is caused by a direct action of gastrin on the hepatocytes or it is an indirect effect mediated by for instance insulin, glucagon or epidermal growth factor has to be further investigated.

In rats partial hepatectomy, with removal of approximately 70% of the liver, induces a rapid induction of cell proliferation in the remaining hepatic tissue.¹ The increase in liver DNA synthesis after partial hepatectomy is initiated after a latent period of 10-14 hours.² Within 10-14 days the liver has regained its original size³ and the regenerative process is terminated abruptly. Experiments in partially hepatectomised rats have shown that removal of portal splanchnic organs, or diversion of portal blood supply delay liver regeneration.⁴⁻⁶ The hepatotrophic factors in portal venous blood may comprise hormones, regulatory peptides, and metabolites.⁷ Insulin and glucagon are important hepatotrophic factors.⁸ Epidermal growth factor also seems to be involved.^{9,10} One study has suggested that gastrin might also be a hepatotrophic factor,¹¹ and increased concentration of gastrin occurs in patients with liver failure.¹²

Gastrin is mainly produced in the antral portion of the stomach.¹³ The peptide is a physiological stimulator of gastric acid secretion and has a trophic effect on parts of the gastrointestinal tract.¹⁴⁻¹⁸

The purpose of this study was to evaluate the role of gastrin on liver regeneration after partial hepatectomy in rats. The concentration of gastrin in portal venous blood after partial hepatectomy was measured and the effect of chronic endogenous hypo- and hypergastrinaemia

as well as administration of pentagastrin on liverweight and DNA content was determined.

Methods

EXPERIMENTAL PROCEDURE

The studies were made on male Wistar rats weighing 300-350 g. The rats were allowed free access to food and water.

Partial hepatectomy was performed according to the method of Higgins³ by removal of the median and left lateral lobes (approximately 70% of the liver). The rats were anaesthetised by intraperitoneal injection of 50 mg/kg methohexital (Brietal®, Lilly, USA).

The effect of partial hepatectomy on the level of gastrin in portal venous blood was investigated in rats in groups of eight. Controls in groups of eight were sham-operated. All rats had blood drawn from the portal vein after 6, 12, 18, 24, 36, 72, and 120 hours, whereafter the rats were killed and the total body weight and the weight of the liver remnant was determined.

The effect of changes in the endogenous gastrin level on liver regeneration after partial hepatectomy was investigated in antrectomised and fundectomised rats. Ten rats underwent antrectomy and nine rats fundectomy as described elsewhere.¹⁸ Controls were sham operated. The rats were fasted 24 hours after surgery, but had free access to water. Three weeks after gastric surgery all rats had a blood sample taken from the inferior vena cava for determination of gastrin, and a 70% partial hepatectomy was performed. Ninety six hours later another blood sample was taken from the inferior vena cava for determination of gastrin, the rats were killed and the total body weight and the weight of the liver remnant was determined. Liver specimens were removed for determination of DNA.

The effect of pentagastrin (Peptavlon®, ICI, England) on liver regeneration after partial hepatectomy was investigated in rats. After partial hepatectomy 18 rats received pentagastrin 300 µg/kg sc three times daily. Eight rats were killed after 48 hours and 10 rats after 96 hours of treatment. Two groups of eight sham operated rats served as controls and received saline. All rats had total body weight and weight of the liver remnant determined. Liver specimens were removed for later determination of DNA.

LABORATORY ANALYSES

Serum gastrin concentration was measured radioimmunochemically. Antiserum 2604 is highly specific for gastrin and binds the three

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larger molecular forms of gastrin with almost equimolar potency,¹⁹ it crossreacts less than 0.1% with cholecystokinin and does not react with other peptides.²⁰ Synthetic human gastrin I was used for monoiodination and standard. The reliability of the assay has been described elsewhere,²¹ as well as the accuracy of the assay in measuring serum from the rat.²²

The DNA content of the liver was measured as described elsewhere.²³

STATISTICAL ANALYSIS
For statistical evaluation the Mann-Whitney's test was used. Analysis of variance was made by Kruskal-Wallis test. Values of $p < 0.05$ were considered significant. All results are given as medians and total ranges.

Results
The concentration of gastrin in portal venous blood and the changes in liver weight after partial hepatectomy are shown in the Figure. A significant rise in serum gastrin concentration was found 6, 12, and 18 hours after partial hepatectomy compared with controls.

The effect of antrectomy and fundectomy on the endogenous gastrin level is shown in Table I. Antrectomy decreases the serum concentration of gastrin by approximately 50%, whereas fundectomy increases the concentration approximately four times. No significant difference in serum gastrin concentration was found in each single group before and four days after partial hepatectomy. Fundectomy had no significant effect on liver regeneration after partial hepatectomy, whereas antrectomy reduced liver regeneration significantly compared with controls (Table II).

Administration of pentagastrin 300 µg/kg sc three times daily, increased the DNA content of the liver remnant significantly 48 and 96 hours

after partial hepatectomy compared with controls (Table III).

TABLE I Serum gastrin concentration three weeks after antrectomy or fundectomy and 96 hours after 70% partial hepatectomy in rats

Treatment	3 weeks gastrin pmol/l	Treatment	96 hours gastrin pmol/l
Sham-operation	74 (44-162)	hepatectomy	74 (24-108)
Antrectomy	25 (13-70)*	hepatectomy	35 (19-110)
Fundectomy	292 (140-482)†	hepatectomy	360 (224-498)†

Results are given as medians and total ranges. * $p < 0.001$ compared with controls; † $p < 0.001$ compared with controls.

TABLE II Effect of antrectomy and fundectomy on liver regeneration after 70% partial hepatectomy in rats

Treatment	n	mg liver DNA/100 g bw
Sham operation + hepatectomy	10	6.75 (5.16-7.94)
Antrectomy + hepatectomy	10	4.93 (4.07-5.52)*
Fundectomy + hepatectomy	9	6.15 (4.43-7.87)

Results are given as medians and total ranges. * $p < 0.005$ compared with controls. All the rats were subjected to 70% partial hepatectomy three weeks after gastric surgery. All the rats were killed 96 hours after partial hepatectomy and the total body weight and the weight, and the DNA content, of the liver remnant were determined.

TABLE III Effect of pentagastrin on liver regeneration after 70% partial hepatectomy in rats

Treatment	Treatment (h)	n	mg liver DNA/100 g bw
Saline	48	8	3.72 (3.15-4.30)
Pentagastrin	48	8	5.60 (4.45-6.14)*
Saline	96	8	6.39 (5.60-6.52)
Pentagastrin	96	10	9.95 (7.48-14.26)†

Results are given as medians and total ranges. * $p < 0.005$ and † $p < 0.005$ compared with controls. Pentagastrin was administered in a dose of 300 µg/kg sc three times daily. Controls received saline three times daily. All rats were killed at the end of treatment and total body weight and weight, and DNA content, of the liver remnant was determined.

Discussion
During the last years evidence has accumulated

Figure: Concentration of gastrin in portal venous blood and change in liver weight after 70% partial hepatectomy in rats. The values shown are the medians. * $p < 0.05$ compared with sham-operated controls.

that humoral factors in portal venous blood are involved in stimulation of liver regeneration after partial hepatectomy in rats.⁷ Insulin, glucagon and epidermal growth factor seems to belong to a group of substances that stimulate liver regeneration.⁸⁻¹⁰

Gastrin has at least two physiological actions: it stimulates gastric acid secretion and has a trophic effect on the gastrointestinal tract. Gastrin stimulates DNA, RNA, and protein synthesis of the mucosa along the entire length of the gut with the exception of the oesophagus and antrum.²⁴

In the present study a possible role of gastrin in stimulation of liver regeneration after partial hepatectomy in rats was demonstrated. The concentration of gastrin in portal venous blood increased 6, 12, and 18 hours after the operation. Whether this is necessary to stimulate liver regeneration or it represents a secondary change in the metabolism of gastrin is unknown. Partial hepatectomised rats develop a characteristic change in the level of several regulatory peptides such as insulin, glucagon, and thyroid hormones, however, substances that are believed to be involved in the stimulation of liver regeneration. These hormonal changes occur rapidly after liver resection, but several hours before the DNA replication starts.^{8, 25, 26} An identical delay has been observed between administration of gastrin and increase in thymidine uptake in mucosa of the intestinal mucosa.¹⁷ The increased level of gastrin in portal venous blood might participate in the initiation of the regenerative process in the liver after partial resection.

In order to show that gastrin physiologically influences liver regeneration in rats one must show the effect of the endogenous hormone. The level of endogenous gastrin can be manipulated by removal of the rat antrum and by fundectomy.¹⁸ We found antrectomy to decrease the level of gastrin by a factor of 2, while the concentration of gastrin after fundectomy increased four to five times. In antrectomised rats liver regeneration, measured by DNA content, decreased significantly, whereas fundectomy had no effect. Antrectomy has previously been shown to induce atrophy of the colonic, duodenal and oxyntic gland mucosa, whereas the weight of the intact liver was unchanged.²⁷ Oscarson *et al.*¹⁸ found fundectomy to increase the level of circulating gastrin, but changes in the height and weight of the gastrointestinal mucosa could not be identified. In rats, made chronic hypergastrinaemic by implanting the gastric antrum into the side of the colon, an increased weight of the intact liver was found.¹¹ The effect of antrectomy in our study suggests that the increase in circulating gastrin after partial hepatectomy is necessary to accelerate liver regeneration. The increase in endogenous serum gastrin concentration after fundectomy is probably too low to accelerate the spontaneous liver regeneration compared to the effect of pentagastrin. One possibility is that antrectomy and fundectomy influence the secretion of various gastrins with different trophic potency²⁸ or other hepatotrophic factors such as insulin and epidermal growth factor.

Pentagastrin has a trophic effect on the gut similar to gastrin 17 and gastrin 34.²⁸ In previous studies the effect of pentagastrin on liver growth has only been investigated on intact livers. Pentagastrin given for 24 hours as well as seven days had no effect on liver growth.^{27, 29} We found a significant effect of pentagastrin on liver regeneration after partial hepatectomy 48 and 96 hours after the operation. The different effect of pentagastrin on the intact and partial hepatectomised liver could be caused by the fact that the growth stimulus elicited by pentagastrin alone is too small to be detected in the intact liver. In the partial hepatectomised rat several other hepatotrophic factors are activated and can act together with pentagastrin such as insulin and glucagon.

In conclusion this study suggests that gastrin is involved in stimulation of liver regeneration after partial hepatectomy in rats. Whether this effect is the result of a direct effect of gastrin on the liver cells or it is an indirect effect mediated through other hepatrophic factors is still unknown.

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