A Unique Surgical Model for Studying the Physiology of Gastrin: Gastrocystoplasty and Fundectomy

by ***RA Vigen[1], C-J Arum[1,2], B Stenström[1] & D Chen[1*]***

[1] Department of Cancer Research and Molecular Medicine,
Norwegian University of Science and Technology, Trondheim, Norway

[2] Department of Surgery, St. Olav's Hospital, Trondheim, Norway

Summary

Gastrin is well known as a gastric acid secreting agent and trophic factor, but the complexity and plasticity of the mechanisms behind its effects need elucidation. For instance, whether the effects depend on vagal innervation is still an open question. In the present report, we describe in technical detail a rat model of gastrocystoplasty and fundectomy with the hope that it will provide an additional tool in gastrin research and an example of experimental surgery.

Introduction

Since JS Edkins discovered gastrin 100 years ago, the physiological significance of this hormone has been studied extensively (for a recent review, see *Dockray et al., 2005*). Gastrin is synthesized and released from the G cells in the antrum. It acts on gastrin/cholecystokinin2 (CCK_2) receptors to stimulate gastric acid secretion and to exert a trophic effect in the oxyntic mucosa (*Koh & Chen., 2000; Lindström et al., 2001; Ohning et al., 1998; Waldum et al., 1991*). Despite extensive studies on gastrin (see, for example: *Merchant et al., 2004; Walsh, 1993*), many questions remain about its physiology, including questions about its interaction with the vagal system.

The importance of vagal innervation in regulating acid secretion has been illustrated by the observation that rats subjected to vagal denervation (vagotomy or treatment with atropine or pirenzepine) exhibit a markedly reduced basal acid output and an abolished acid response to all stimuli (*Ekelund et al., 1987; Håkanson et al., 1982; Vallgren et al., 1983*). Why vagotomy and atropine block the acid response to gastrin (and histamine) is unclear. Vagotomy also suppressed the trophic effect of gastrin in the oxyntic mucosa, including the enterochromaffin-like (ECL) cells (*Axelson et al., 1988; Håkanson et al., 1992*). A recent study found impaired acid secretion and no trophic responses in the oxyntic mucosa in M_3 muscarinic receptor knockout mice, which were remarkably hypergastrinemic, suggesting that M_3 receptors are essential for acid secretion and for the trophic responses to gastrin (*Aihara et al., 2003*).

The interaction between gastrin and the vagal system has been studied using isolated parietal or ECL cells, isolated oxyntic glands, and totally isolated vascularly perfused stomachs. Studies using isolated mouse or rat stomach showed that electrical stimulation of vagal fibers activated cholinergic excitatory pathways and noncholinergic inhibitory pathways to the gastric glands (*Davison & Najafi-Farashah, 1987; Sandvik & Waldum, 1991*). In rats or mice, vagotomy or atropine could abolish the acid response to vagal excitation evoked by pylorus ligation (*Håkanson et al., 1980; Noto et al., 1997; Chen et al., 2004*). A recent study of gastrin and CCK double-knockout mice indicated that without

**Correspondence:* D Chen, Professor, MD, PhD
Department of Cancer Research and Molecular Medicine,
Laboratory Center of St. Olavs Hospital, Erling
Skjalgssons Gate 1, NO-7006 Trondheim, Norway.
Tel: +47 72 57 33 20
Fax: +47 72 57 64 12/64 11
E-mail: duan.chen@ntnu.no

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

the endocrine and paracrine pathways (e.g., gastrin–histamine and CCK–somatostatin), neural (vagal) pathways might take over control of acid secretion and the trophic effect (*Chen et al., 2004*). Carbachol (an M_3 receptor agonist) stimulated acid secretion in wild-type mice, but reduced it in the gastrin–CCK double knockout mice, suggesting that the mechanisms controlling acid secretion in wild-type mice differ from those controlling acid secretion in the double knockouts (*Chen et al., 2004*). Taken together, these data suggest that there may be suppression or dominance of the gastrin pathway depending on the circumstances and that the nature and precise role of the neural pathway in regulating acid secretion remains to be defined.

Here we employed a rat model of gastrocystoplasty, which has been used to improve capacity and compliance in patients with bladder dysfunction (*Kurzrock et al., 1998; Leong, 1978*), to study whether gastrin alone can stimulate acid secretion. In our recent study, the rats did not produce measurable acid in the bladder when subjected to gastrocystoplasty alone; however, when they were subjected to both gastrocystoplasty and fundectomy (known to evoke hypergastrinemia via a feedback loop of gastric acid–gastrin), the rats secreted acid in the bladder in response to food intake (*Arum et al., 2005*). In the present report, we describe the experimental model of gastrocystoplasty and fundectomy in details of the surgical technique with the hope that it will serve as an additional model in the field of gastrin research.

Material and Methods

Animals

Male Sprague-Dawley rats were purchased from Möllegaard (Skensved, Denmark) and housed in plastic cages (55 x 33 x 19 cm) (Scanbur BK, Karlslunde, Denmark) with hardwood chips (BeeKay bedding, aspen wood chips; Scanbur BK). Room temperature was 22 ±1 ° C, with a relative humidity of 45%–55% and 12-h light/dark cycle. The rats had free access to commercial standard rat food pellets (RM1, Scanbur BK AS, Denmark) and tap water *ad libitum*. Microbiological status was conventional and pathogen free. There was no preoperative fasting, and no antibiotic prophylaxis was given. Approval for the experiments was obtained from The Norwegian Animal Research Authority (Forsøksdyrutvalget, FDU).

Anesthesia

Rats were anesthetized for the operation with an intraperitoneal injection of a solution of Equitesin (21.3 g chloral hydrate, 10.6 g magnesium sulfate, 4.8 g Na pentobarbital, 50.0 g 96% ethanol, 200 g propylene glycol, and Aqua purificata 213.3 ml distilled water). After the rat was anesthetized, its abdomen was shaved with a hairclipper, and it was placed in the supine position on a heated (~37 °C) operation table.

For an overview of surgical equipment and disposables used, see Table 1.

Gastrocystoplasty

A segment of the oxyntic mucosa (i.e., the fundus in the rat) of the stomach was used. The oxyntic mucosa is the special target of gastrin (*Koh & Chen, 2000*). It is well known that vagal intervention at this location occurs through both the anterior and posterior vagal trunks. Therefore, the transplanted oxyntic mucosa in the bladder after gastrocystoplasty is vagotomized, although the vascular pedicle may carry other nerves.

A longitudinal midline incision was made from the xiphoid process towards the symphysis pubis, and the gastric patch was isolated along with its vascular pedicle. The right gastroepiploic artery was chosen to supply the patch, and the left gastroepiploic artery was ligated using a 7-0 nonabsorbable monofilament suture (Prolene®, Ethicon Inc., Somerville, NJ, USA). The greater omentum was then fenestrated with a curved forceps at each end of the anticipated gastric patch in order to ligate the branches from the right gastroepiploic artery. A segment approximately 7 mm in diameter composed of anterior and posterior full-thickness stomach wall, centered on the greater curvature, was iso-

Table 1: Surgical equipment and articles of consumption disposables

Surgical equipment

- Standard needle holder
- Microsurgical needle holder
- Tissue forceps
- Several multi-purpose hemostat artery forceps
- "deBakey" A traumatic tissue forceps, e.g. de Bakey
- Scalpel or a blunt-ended pair of scissors
- Small tissue scissors
- Microscope or microscopic glasses

Other equipment

- Operating table with adjustable temperature
- Digital Timer
- Box for used cannulas
- Fiberoptic halogen spot-lighting
- Heating lamp
- Heated cage for post-operative recovery (e.g. Scanbur BK AS, Denmark)
- Glass bead sterilizer (Steri 250)
- Inotech Biosystems International, Inc) for sterilizing instruments prior to surgery
- Digital temperature monitor (e.g. Physiotemp)

Disposables

- Nonabsorbable polypropylene monofilament sutures (e.g. Prolene 7-0),
- Absorbable coated braided suture (e.g. Vicryl 4-0)
- Saline 0.9%
- Chlorhexidine
- Simplex ointment (20% paraffin, 80% Vaseline) for eye protection
- 22 gauge cannulas
- 10-ml syringes for subcutaneous post-opertive saline injections
- 2-ml syringes for catheterizations
- 1-ml syringes for medications
- Disinfection: 75% alcohol solution

lated with clamps and excised with an intact vascular pedicle (Fig. 1).

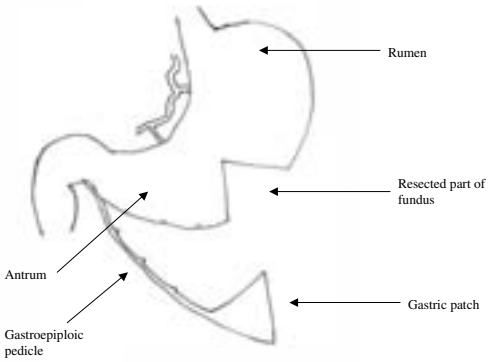

Figure 1: Graphic illustrating of isolation of the gastric patch

The clamps were left in place until thebladder augmentation was complete (see *Fundectomy and pyloroplasty* below). The elliptical gastric patch with the vascular pedicle, attached to the greater omentum, was then easily mobilized to the lower abdominal cavity without tension. The augmentation was constructed as follows: the bladder fundus was first fixed with two 7-0 holding sutures and then opened with an approximately 5–6 mm transverse incision. The gastric patch was sutured to the bladder using a 7-0 absorbable monofilament suture with a continuous running inverted suture technique (Fig. 2), and the holding sutures were

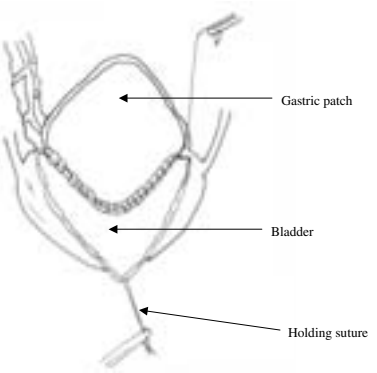

Figure 2: Graphic illustrating of bladder augmentation

then removed. The integrity of the augmentation was tested by catheterizing the rat, using a 22-gauge cannula to fill the bladder with 1 ml 0.9% sterile saline. Any leakage in the anastomosis was reinforced with additional suture(s).

For performing gastrocystoplasty alone, the clamps were removed, and the stomach was closed with a running layer of continuous nonabsorbable 7-0 suture.

Fundectomy and pyloroplasty

As described above, the gastrocystoplasty with the segment (7 mm in diameter) of the fundus represents a < 10% fundectomy. It has been found that serum gastrin concentration will not increase significantly until 50% of the fundus is removed (*Lehto-Axtelius et al., 2002*). Complete fundectomy has been used as a model to study the effects of hypergastrinemia the pancreas (*Chen et al., 1996*). We have found that the hypergastrinemia response was similar in fundectomies between 90% and 100% (*Lehto-Axtelius et al., 2002*). Here, we recommend performing the 90% fundectomy in addition to gastrocystoplasty; this approach allows study of the oxyntic mucosa that remains in the stomach which is vagally innervated, while that transplanted to the bladder is not.

After augmentation was complete, a 90% fundectomy was performed, keeping a thin brim of oxyntic mucosa next to the rumen. The clamps had been applied to the stomach to prevent bleeding when resecting the gastric patch. These were now removed with insignificant bleeding and applied along the easily identified borders dividing the fundus and antrum and the fundus and rumen. About 10% of the oxyntic mucosa (*Lehto-Axtelius et al., 2002*) was retained after resection along the clamps. By applying holding sutures at the greater and lesser curvatures and releasing the forceps, being careful not to contaminate the abdominal cavity with gastric contents, the front of the rumen and antrum was joined with a single layer of running nonabsorbable 7-0 suture. Pulling one of the holding sutures underneath the stomach allowed for turning of the stomach, exposing the unsewed backside. The suture was continued along the backside, and the anastomosis was controlled by gentle pinching of the stomach after closure. Contamination of the abdominal cavity was evaluated before rinsing with 0.9% saline.

To prevent gastroparesis resulting from the cutting of vagal fibers towards the pylorus after fundectomy, the Heineke-Mikulicz pyloroplasty was also performed. A ~5-mm longitudinal incision (in relation to the intestine) was made through the pylorus muscle, which was carefully divided; diathermia was used to stop potential bleeding, and the muscle ends were cauterized. The incision was then closed transversally with a continuous single layer of nonabsorbable 7-0 monofilament suture (Fig. 3). The abdominal wall was closed with a continuous 4-0 absorbable braided suture (Vicryl®, Ethicon Inc., Somerville, NJ, USA) in the rectus fascia, and the skin also was closed with this suture.

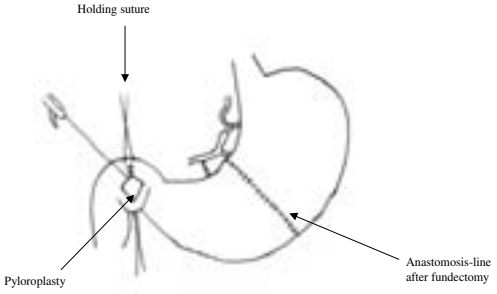

Figure 3: Graphic illustrating of fundectomy and pyloroplasty

Postoperative care

Fluid balance was maintained with a subcutaneous injection of 25 ml/kg 0.9% NaCl, given immediately after surgery. Postoperative analgesia was accomplished with 0.05-mg/kg buprenorphine (Temgesic® 0.3 mg/ml) given subcutaneously, 1–2 times/day (it lasts 8–12 h) for 2–3 days. For recovery, the rats were placed separately in a heating cage (Scanbur BK, Denmark) set to an air temperature of 30–35 °C for 2–3 hours. For longer recovery times, physiological saline was given subcutaneously. Rats

were then placed overnight in separate cages with free access to tap water and food pellets. After complete recovery, they were put together in preoperative housing groups. Body weights were recorded daily during the first week to identify potential severe complications. Prolonged decline and delayed regain were handled by repeated saline and analgetic administration. Body weights were then recorded weekly through the follow-up period (Fig. 4).

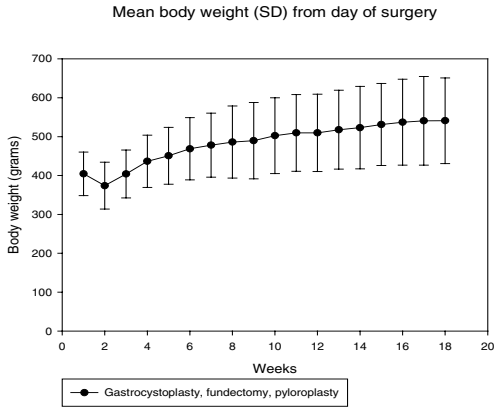

Figure 4: Body-weight gain in rats subjected to gastrocystoplasty, fundectomy, and pyloroplasty

Surgical experiences and follow-up

Based on averages from 40 operated rats, performing the gastrocystoplasty took approximately 30 minutes, and an additional 25 minutes were required for performing the fundectomy with pyloroplasty. In general, body weights dropped during the first postoperative week, especially after fundectomy, but by the sixth week the operated animals had almost regained weight compared with the non-operated controls. Peri- and postoperative mortality rates were between 0%–10% for all surgeries. After gastrocystoplasty, urinary output was ~10–15 ml/rat/24 h, and water intake was ~20–30 ml/rat/24 h. There were no significant differences in urinary output or water intake between the normal or sham-operated rats and the rats subjected to gastrocystoplasty (with or without fundectomy).

Acknowledgements

This work was supported by a grant from the Cancer Foundation of St. Olav's Hospital, Trondheim, Norway. Reidar Alexander Vigen is recipient of a medical student summer-scholarship from the Faculty of Medicine, Norwegian University of Science and Technology, and a semester scholarship from the Norwegian Research Council.

References

Aihara T, T Fujishita, K Kanatani, K Furutani, E Nakamura, MM Taketo, M Matsui, D Chen, & S Okabe: Impaired gastric secretion and lack of trophic responses to hypergastrinemia in M3 muscarinic receptor knockout mice. Gastroenterology, 2003, *125*, 1774-1784.

Arum C-J, RA Vigen, B Karlsson, K Tømmerås, CM Zhao & D Chen: Acid secretion in urinary bladder of rats subjected to gastrocystoplasty. Inflammopharmacology, 2005, *13* (9), 191-199.

Axelson J, M Ekelund, R Håkanson, & F Sundler: Gastrin and the Vagus Interact in the Trophic Control of the Rat Oxyntic Mucosa. Regul. Pept., 1988, *22*, 237-243.

Chen D, CM Zhao, R Håkanson, LC Samuelson. JF Rehfeld, & L Friis-Hansen: Altered control of gastric acid secretion in gastrin-cholecystokinin double mutant mice. Gastroenterology, 2004, *126*, 476-487.

Chen D, AG Nylander, P Norén, & R Håkanson: Gastrin does not stimulate growth of the rat pancreas. Scand. J. Gastroenterol., 1996, *31*, 404-10.

Davison JS & A Najafi-Farashah: Vagal inhibition of gastric acid secretion: evidence for cholecystokinin as the inhibitory transmitter in the mouse stomach. Can. J. Physiol Pharmacol, 1987, *65*, 1937-1941.

Dockray G, R Dimaline, & A Varro: Gastrin: old hormone, new functions. Pflugers Arch, 2005, *449*, 344-355.

Ekelund M, R Håkanson, & S Vallgren: Effects of cimetidine, atropine and pirenzepine on basal

and stimulated gastric acid secretion in the rat. Eur. J. Pharmacol, 1987, *138*, 225-232.

Håkanson R, J Axelson, Y Tielemans, AG Johansson, G Willems, & F Sundler: Unilateral vagal denervation suppresses omeprazole-induced trophic effects on the denervated side of the rat stomach. Scand. J. Gastroenterol., 1992, *27*, 65-70.

Håkanson R, J Hedenbro, G Liedberg, F Sundler & S Vallgren: Mechanisms of gastric acid secretion after pylorus and oesophagus ligation in the rat. J. Physiol., 1980, *305*, 139-49.

Håkanson R, J Hedenbro, G Liedberg, & S Vallgren: Effect of vagotomy on gastric acid secretion in the rat. Acta. Physiol. Scand., 1982, *115*, 135-139.

Koh TJ & D Chen: Gastrin as a growth factor in the gastrointestinal tract. Regul. Pept., 2000, *93,* 37-44.

Kurzrock EA, LS Baskin, & BA Kogan: Gastrocystoplasty: long-term followup. J. Urol, 1998, *160*, 2182-2186.

Lehto-Axtelius D, D Chen, VV Surve, & R Håkanson: Post-gastrectomy osteopenia in the rat: bone structure is preserved by retaining 10%-30% of the oxyntic gland area. Scand. J. Gastroenterol., 2002, *37*, 437-443.

Leong CH: Use of the stomach for bladder replacement and urinary diversion. Ann. R. Coll. Surg. Engl., 1978, *60*, 283-289.

Lindström E, D Chen, P Norlen, K Andersson, & R Håkanson: Control of gastric acid secretion:the gastrin-ECL cell-parietal cell axis. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 2001, *128*, 505-51.

Merchant JL, AMJ Buchan, TC Wang (eds.): Gastrin in the New Millennium. Cure Foundation, Los Angeles, USA, 2004.

Noto T, M Nagasaki, & T Endo: Role of vagus nerves and gastrin in the gastric phase of acid secretion in male anesthetized rats. Am. J. Physiol., 1997, *272,* G335-9.

Ohning GV, M Song, HC Wong, SV Wu, & JH Walsh: Immunolocalization of gastrin-dependent histidine decarboxylase activity in rat gastric mucosa during feeding. Am. J. Physiol., 1998, *275*, G660-G667.

Sandvik AK & HL Waldum: Aspects of the Regulation of Gastric Histamine-Release. Scand. J. Gastroenterol., 1991, *26*, 108-112.

Vallgren S, M Ekelund, & R Hakanson: Mechanism of inhibition of gastric acid secretion by vagal denervation in the rat. Acta. Physiol. Scand., 1983, *119*, 77-80.

Waldum HL, AK Sandvik, E Brenna & H Petersen: Gastrin-histamine sequence in the regulation of gastric acid secretion. Gut, 1991, *32*, 698-701.

Walsh JH: Gastrin. Raven Press, New York, USA, 1993.