British Journal of Pharmacology (2000) 130, 725–730
www.nature.com/bjp

Anaesthetic agents inhibit gastrin-stimulated but not basal histamine release from rat stomach ECL cells

[1]P. Norlén, [1]M. Kitano, [1]E. Lindström & *,[1]R. Håkanson

[1]Department of Pharmacology, Institute of Physiological Sciences, University of Lund, Sölvegatan 10, Lund, Sweden

1 By mobilizing histamine in response to gastrin, the ECL cells in the oxyntic mucosa play a key role in the control of the parietal cells and hence of gastric acid secretion. General anaesthesia suppresses basal and gastrin- and histamine-stimulated acid secretion. The present study examines if the effect of anaesthesia on basal and gastrin-stimulated acid secretion is associated with suppressed ECL-cell histamine secretion.

2 A microdialysis probe was implanted in the submucosa of the ventral aspect of the acid-producing part of the stomach (32 rats). Three days later, ECL-cell histamine mobilization was monitored 2 h before and 4 h after the start of intravenous infusion of gastrin (5 nmol kg^{-1} h^{-1}). The rats were either conscious or anaesthetized. Four commonly used anaesthetic agents were given 1 h before the start of the experiments by intraperitoneal injection: chloral hydrate (300 mg kg^{-1}), pentobarbitone (40 mg kg^{-1}), urethane (1.5 g kg^{-1}) and a mixture of fluanisone/fentanyl/midazolam (15/0.5/7.5 mg kg^{-1}).

3 In a parallel series of experiments, basal- and gastrin-induced acid secretion was monitored in six conscious and 25 anaesthetized (see above) chronic gastric fistula rats. All anaesthetic agents lowered gastrin-stimulated acid secretion; also the basal acid output was reduced (fluanisone/fentanyl/midazolam was an exception).

4 Anaesthesia reduced gastrin-stimulated but not basal histamine release by 55–80%. The reduction in gastrin-induced acid response (70–95%) was strongly correlated to the reduction in gastrin-induced histamine mobilization. The correlation is in line with the view that the reduced acid response to gastrin reflects impaired histamine mobilization.

5 Rat stomach ECL cells were purified by counter-flow elutriation. Gastrin-evoked histamine mobilization from the isolated ECL cells was determined in the absence or presence of anaesthetic agents in the medium. With the exception of urethane, they inhibited gastrin-evoked histamine secretion dose-dependently, indicating a direct effect on the ECL cells.

6 Anaesthetized rats are widely used to study acid secretion and ECL-cell histamine release. The present results illustrate the short-comings of such an approach in that a number of anaesthetic agents were found to impair not only acid secretion but also the secretion of ECL-cell histamine – some acting in a direct manner.

British Journal of Pharmacology (2000) **130,** 725–730

Keywords: Anaesthesia; acid output; histamine; microdialysis; ECL cells

Introduction

Histamine in the stomach occurs in mast cells and ECL cells (Håkanson *et al*., 1986; 1994). In the rat, 80–90% of gastric histamine is in ECL cells (Andersson *et al*., 1992; 1996). Unlike mast cells (Soll *et al*., 1988), ECL cells respond to gastrin by mobilization of histamine that in turn stimulates acid secretion from parietal cells (Håkanson & Sundler, 1991; Waldum *et al*., 1991; Andersson *et al*., 1996). This pathway has been referred to as the gastrin-ECL cell-parietal cell axis (Håkanson *et al*., 1999).

General anaesthesia is known to suppress both basal and gastrin- and histamine-stimulated acid secretion (Lee & Thompson, 1967; Barret *et al*., 1978; Graffner *et al*., 1991; Bastaki *et al*., 1995). It is however not known whether this reflects a suppressive effect on parietal cells, vagus/enteric neurons or ECL cells. By the use of *in vivo* microdialysis it has become possible to study the gastrin-evoked mobilization of ECL-cell histamine in conscious rats (Kitano *et al*., 2000). The purpose of the present study was to examine if the suppressive effect of anaesthetic agents on basal and gastrin-stimulated acid secretion was associated with inhibition of ECL-cell histamine secretion. The question whether anaesthetic agents are capable of acting directly on the ECL cells was studied using isolated rat stomach ECL cells.

*Author for correspondence: E-mail: Rolf.Hakanson@farm.lu.se

Methods

Chemicals

The isolated ECL cells were cultured in 96-well plates coated with Matrigel®(Collaborative Biomedical Products, Bedford, MA, U.S.A.) diluted 1 : 10 with Dulbecco's modified Eagle's medium (DMEM)- Ham's F12 (Sigma, St. Louis, MO, U.S.A.). Rat gastrin-17 was purchased from Research Plus, Bayonne, NJ, U.S.A. Chloral hydrate was obtained from Acros, Geel, Belgium, pentobarbitone from Apoteksbolaget, Umeå, Sweden and urethane from ICN, Aurora, OH, U.S.A. A stock solution of fluanisone/fentanyl (HypNorm®) (Janssen, Beerse, Belgium) and midazolam (Dormicum®) (Roche, Basel, Switzerland) was freshly prepared for each experiment. HypNorm® and Dormicum® were diluted with equal volumes of saline and mixed (1 : 1). Final concentrations: 15 (fluanisone), 0.5 (fentanyl) and 7.5 (midazolam) mg ml^{-1}.

Animals

Male Sprague-Dawley rats (250–300 g) were kept at a 12-h light and 12-h dark cycle in plastic cages (4–6 in each cage) with free access to standard rat food pellets (B&K Universal, Sollentuna, Sweden) and tap water. During fasting, they were housed in individual cages with wire mesh bottoms for 48 h without food but with free access to water. During the experiments, the rats were kept in Bollman-type restraining cages. Starting 2 weeks prior to the experiments all rats had been thoroughly familiarized with the Bollman cages by daily training for 1–2 h. The rats were fitted with microdialysis probes on the ventral aspect of the acid-producing part of the stomach (see below) 3 days before the experiments. A plastic catheter was inserted into the right jugular vein at the same time. Surgery was performed under chloral hydrate anaesthesia (300 mg kg^{-1} intraperitoneally). The studies were approved by the local Animal Welfare Committee, Lund/Malmö.

In vivo *microdialysis*

A flexible microdialysis probe (MAB3.8.10, AgnTho's AB, Stockholm, Sweden, length 10 mm, outer diameter 0.57 mm, 35 kDa cut-off) was used. Briefly, the microdialysis probe was inserted into the submucosa of the ventral aspect of the acid-producing part of the stomach and kept in place with sutures. The inlet and outlet tubes were passed through the abdominal opening and tunnelled under the skin to a point at the nape of the neck (for details see Kitano *et al*., 2000). Sampling of microdialysate was performed 3 days after the implantation of the microdialysis probe. The inlet tube was connected to a microinfusion pump (Model 361, Sage instrument, ATI Orion, Boston, U.S.A.) and the outlet was allowed to drain into 300 μl polypropylene vials. Perfusion of the microdialysis probes with degassed saline (1.2 μl min^{-1}) started at 07.00 h. Rats were given saline intravenously at a rate of 1 ml h^{-1} during the 2 h of basal microdialysate sampling (one sample per hour). Synthetic human Leu^{15}-gastrin-17 (in 0.9% saline enriched with 1% bovine serum albumine) was then infused intravenously at a dose of 5 nmol kg^{-1} h^{-1} for 4 h (1 ml h^{-1}). Microdialysis samples were collected every 20 min during the first hour and then every hour. Each rat and each microdialysis probe was used once only. Histamine in the microdialysates was determined by radioimmunoassay using a commercially available kit (Immunotech, Marseilles, France). The histamine concentration was expressed as nmoles per liter microdialysate or pmoles per 4 h.

Chronic gastric fistula

A chronic gastric fistula was implanted in the rumen close to the acid-producing part of the stomach (Bel *et al*., 1966). The rats were allowed to recover for 2 weeks before the experiments. On the day of the experiment, the fasted rats were put in Bollman cages and the stomachs were washed with 3×20 ml of tepid 0.9% saline and drained for 1 h. Rats received saline intravenously at a rate of 1 ml h^{-1} during 1 h of draining and 2 h of basal collection (one sample per hour). Synthetic human Leu^{15}-gastrin-17 was then infused intravenously (5 nmol kg^{-1} h^{-1}, 1 ml h^{-1}). Gastric juice was collected (free drainage) through the fistula every 20 min during the first hour and then every hour. Acid output was determined by titration with 20 mM NaOH to pH 7.0 using an automatic titration assembly (Metrohm Herisau, Switzerland). Acid secretion was expressed as μmoles H^+ per hour or μmoles H^+ per 4 h.

Design of study: histamine and gastric acid secretion

Basal microdialysate samples or gastric juice were collected during 2 h, followed by collection of further samples during intravenous infusion of gastrin for 4 h. The rats were either awake or anaesthetized. Four different anaesthetic agents were selected on the criteria that they have been used in previous studies of ECL cells histamine secretion or gastric acid secretion: chloral hydrate (300 mg kg^{-1}) (Graffner *et al*., 1991), pentobarbitone (40 mg kg^{-1}) (Gerber & Payne, 1992; Aurang *et al*., 1997), urethane (1.5 mg kg^{-1}) (Watanabe *et al*., 1987; Kitamura *et al*., 1999) and a mixture of fluanisone/fentanyl/midazolam (15/0.5/7.5 mg kg^{-1}, 1 mg kg^{-1}) (Mårvik *et al*., 1995). The drugs were given by intraperitoneal injection 1 h before collection of basal acid or microdialysate histamine. The depth of the anaesthesia was monitored by testing eyelid reflexes. When rats responded to stimulation, an additional 30% of the original dose was given by intraperitoneal injection. Usually two additional injections had to be given to rats anaesthetized with chloral hydrate (1 and 2 h after the first injection) while one additional injection was sufficient for the other anaesthetic agents (1 h after the first injection). As a result the rats were under anaesthesia for 7 h. Rats that were conscious throughout the experiment received intraperitoneal injections of 0.9% saline (1 ml). The integrated basal histamine mobilization and basal acid output was calculated by multiplying the total volume of microdialysate (1.2 μl min^{-1}) or basal gastric juice with the mean concentrations of histamine of H^+ respectively during the 2 h of basal sampling (data were taken from Figure 1A,B). The gastrin-evoked acid and histamine responses were calculated by integrating the amounts of H^+ or histamine, respectively, collected during 4 h of gastrin infusion while subtracting the integrated basal values (see above) for the same time period. The results were expressed as the increment (Δ) in H^+ or histamine mobilized during the 4 h stimulation period.

Isolation and primary culture of ECL cells

The ECL cells were purified as described in detail previously (Lindström *et al*., 1997). Briefly, cells were harvested from the oxyntic mucosa of four rats at a time. Repeated counter-flow elutriation resulted in an average yield of 2,000,000 cells. The purity of the ECL cell preparation was assessed by immunocytochemistry (for details see Lindström *et al*., 1997), using a guinea-pig anti-rat histidine decarboxylase antiserum (1 : 750, code no. M9501, Dartsch *et al*., 1999, a kind gift from Dr L. Persson, Department of Physiology, Institute of Physiological Sciences, University of Lund, Sweden) and found to be $80 \pm 5\%$ ($n = 8$). The cells were cultured in 96-well plates pre-coated with Matrigel® (diluted 1 : 10 with DMEM-Ham's F12) (5000 cells per well) in a humid atmosphere with 5% CO_2/95% air at 37°C for 48 h until the start of the experiments. The culture medium consisted of DMEM-Ham's F12 (1 : 1) supplemented with 2% foetal calf serum, glutamine 2 mM, penicillin 100 IU ml^{-1}, streptomycin 100 μg ml^{-1}, amphotericin B 250 ng ml^{-1}, insulin 10 μg ml^{-1}, transferrin 5.5 μg ml^{-1}, selenium 5 ng ml^{-1}, bovine serum albumin 0.5 μg ml^{-1}, hydrocortisone 10 nM, HEPES 15 mM, pyridoxal-5-phosphate 10 μM and, unless otherwise stated, gastrin 0.1 nM.

Histamine secretion from isolated ECL cells in primary culture

After 48 h the culture medium was replaced with fresh serum-free, gastrin-free culture medium. After equilibration for 2 h, the medium was again aspirated and replaced with secretion medium (mM): NaCl 150, KCl 5, $CaCl_2$ 2, HEPES 10 at pH 7.0. Basal and stimulated (10 nM gastrin) histamine secretion was measured (after 30 min incubation) in the absence or presence of increasing amounts of the various anaesthetic agents (for each agent the highest concentration was in the same range as the one used to induce anaesthesia *in vivo* (w v^{-1}). The incubation was interrupted by centrifuging the plates at $220 \times g$ for 1 min and collecting the supernatants for measurement of histamine (as above).

Statistics

Experimental data are expressed as means ± s.e.mean. Differences were statistically analysed by one way analysis of variance (ANOVA) followed by the Dunnett multiple comparison test. The relationship between suppressive effects of anaesthetic agents on basal and stimulated histamine secretion *versus* basal and stimulated acid secretion was quantified by calculating the correlation coefficient (r), using the Pearson correlation (GraphPad Prism version 3.00, GraphPad Software, San Diego, CA, U.S.A.).

Results

Acid output: gastric fistula rats

Basal acid output was reduced to a varying degree by the different anaesthetic agents (Figure 1A). While fluanisone/fentanyl/midazolam failed to reduce the basal acid output, urethane reduced it by 40% and pentobarbitone or chloral hydrate almost abolished it. In conscious rats, infusion of gastrin raised the acid output 4 fold within 1 h, the integrated Δ acid response being 870 ± 150 μmol H^+ during the 4 h stimulation period. Rats anaesthetized with chloral hydrate responded poorly to gastrin (the integrated Δ H^+ being 30 ± 15 μmol), while rats given urethane, pentobarbitone or a

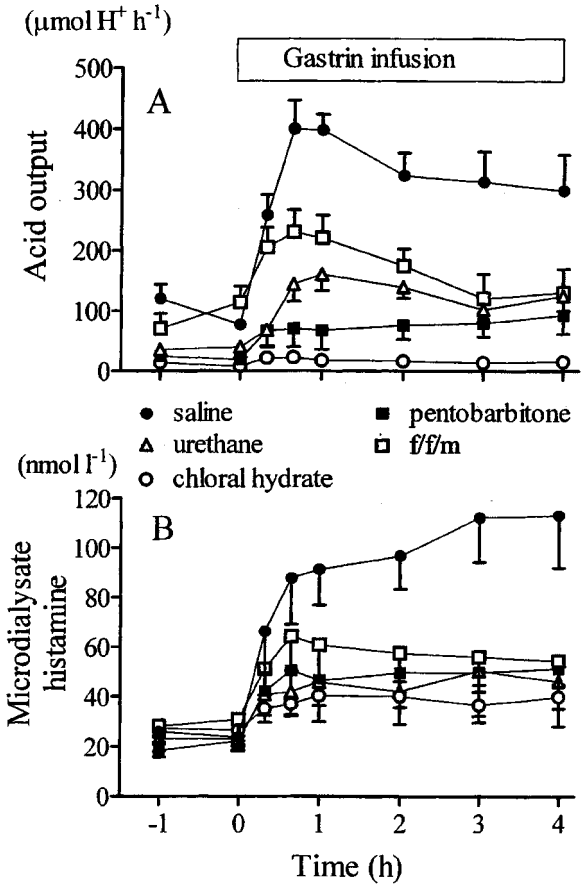

Figure 1 Time course of gastrin-induced acid secretion (A) and rise in microdialysate histamine concentration (B) in conscious and anaesthetized rats. The experiment was conducted as a parallel study of one set of gastric fistula rats (A) and one set of rats implanted with a microdialysis probe in the gastric submucosa (B). The microdialysis probe was inserted 2 days before the experiments. Gastrin was given by continuous intravenous infusion (5 nmol kg^{-1} h^{-1}) (jugular vein), starting at time zero. Means ± s.e.mean (6–8 rats in each group). The mixture of fluanisone/fentanyl/midazolam is abbreviated f/f/m.

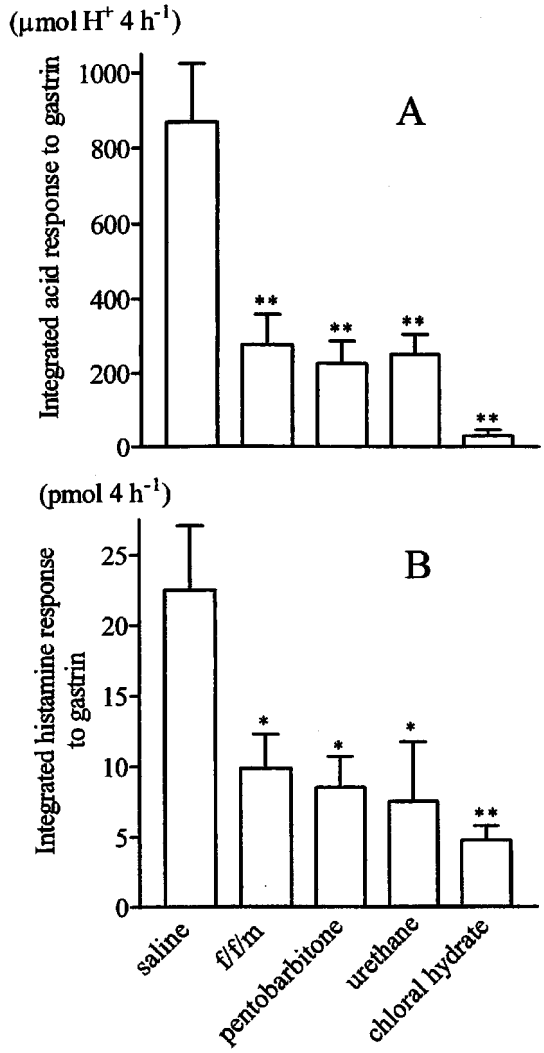

Figure 2 The gastrin-evoked stimulation of acid (A) and histamine (B) secretion in conscious and anaesthetized rats was expressed as the integrated increment (Δ) in H^+ and histamine during 4 h of gastrin stimulation. The increment in response to gastrin was calculated by multiplying the total volume of gastric juice of microdialysate with their respective concentrations of H^+ or histamine, while subtracting the integrated basal secretion during the same time period. f/f/m stands for fluanisone/fentanyl/midazolam. Results are given as mean ± s.e.mean ($n = 6-8$ in each group). Statistical significance was calculated by ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ (*) was considered significant, $P < 0.01$(**).

mixture of fluanisone/fentanyl/midazolam responded with about 30% of the response of conscious rats (Figures 1A and 2A).

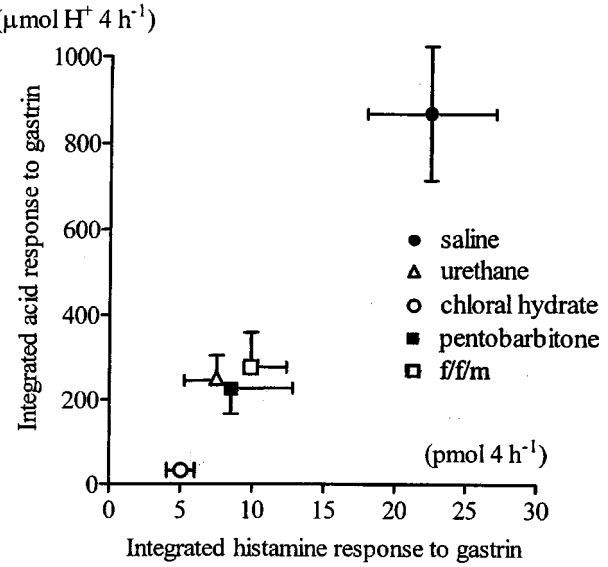

Figure 3 Relationship between acid output and microdialysate histamine in conscious and anaesthetized rats. Data shown are the integrated acid output *versus* the amount of histamine released (calculated from the microdialysate histamine concentration) during 4 h of gastrin infusion (5 nmol kg^{-1} h^{-1}). The increment in acid output or histamine mobilization during 4 h of gastrin stimulation was calculated as described in Figure 2. The relationship between acid output *versus* histamine mobilization was estimated using the Pearson correlation. f/f/m stands for fluanisone/fentanyl/midazolam.

Histamine secretion: in vivo *microdialysis*

Basal histamine secretion was not affected by any of the anaesthetic agents. In conscious rats, gastrin infusion increased the microdialysate histamine concentration 3–4 fold within 1 h (Figure 1B). All anaesthetic agents reduced the response to gastrin. Pentobarbitone, urethane and the mixture of fluanisone/fentanyl/midazolam inhibited the gastrin-evoked histamine release by 55–65%, and chloral hydrate inhibited it by about 80% (Figures 1B and 2B).

Plotting the basal acid output *versus* the amount of basal microdialysate histamine for each of the four anaesthetic agents (or mixture of such agents) revealed that the two parameters were independent of each other ($r^2 = 0.001$) (not shown). In contrast, the reduction in the gastrin-induced acid response was well correlated to the reduction in the gastrin-induced mobilization of histamine ($r^2 = 0.98$) (Figure 3).

Histamine secretion: isolated ECL cells in primary culture

Basal histamine release from isolated ECL cells was 2.5 ± 0.1 ($n = 26$) pmol $well^{-1}$ (the secretion period was 30 min). Histamine secretion with 10 nM gastrin in the medium was 10.3 ± 0.3 ($n = 26$) pmol $well^{-1}$. With the exception of urethane, the anaesthetic agents tested reduced histamine secretion from gastrin-stimulated ECL cells dose-dependently (Figure 4A–D). Chloral hydrate and pentobarbitone reduced histamine secretion from gastrin-stimulated cells by 75–80% (i.e., basal histamine release remaining), and fluanisone/fentanyl/midazolam reduced histamine secretion by 60%. None of the anaesthetic agents affected the viability of the

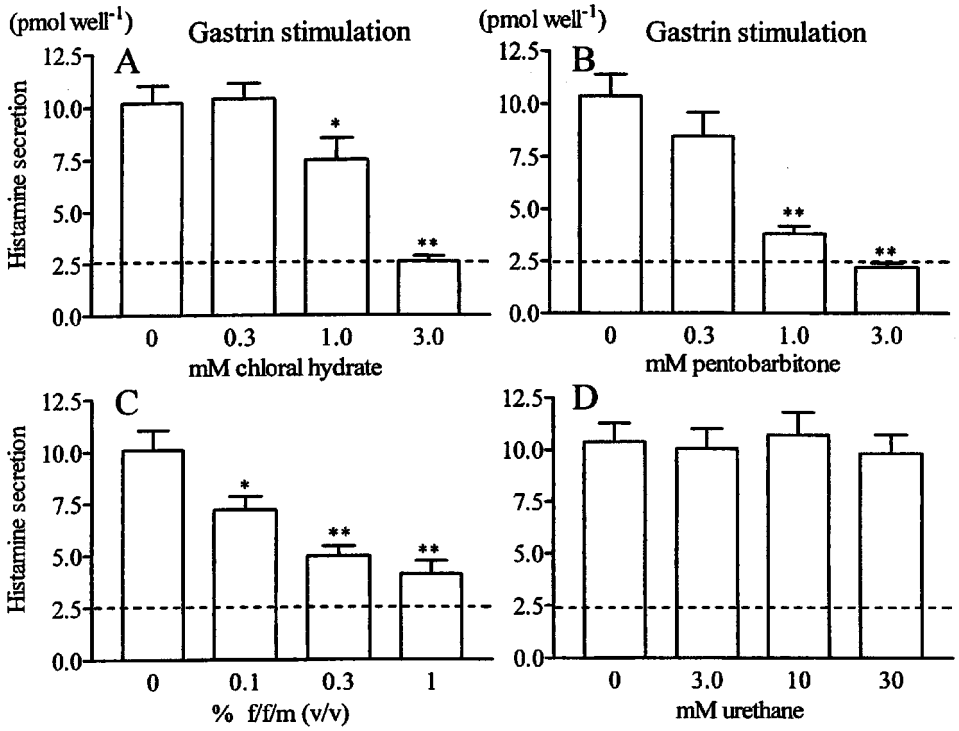

Figure 4 Effects of anaesthetic agents on isolated ECL cells in primary culture. Histamine secretion in response to gastrin (10 nM, 30 min) was determined in the absence or presence of the anaesthetic agents. The basal histamine secretion (no gastrin, 30 min) is indicated as a horizontal line. The final concentrations of the anaesthetic agents in the secretion medium are given in mM except for fluanisone/fentanyl/midazolam (f/f/m, see Methods), which is given in per cent of the stock solution (v v^{-1}). Results are expressed as mean ± s.e.mean ($n = 6–8$ in each group). Statistical significance was calculated by ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ (*) was considered significant, $P < 0.01$ (**).

ECL cells as verified by Trypan Blue exclusion (>95% viability at the conclusion of the experiments).

Discussion

Despite the antisecretagogue effect of general anaesthesia, anaesthetic agents are widely used in studies of gastric acid secretion and histamine mobilization. The inhibitory potential of anaesthetic agents on both basal and gastrin- and histamine-stimulated acid secretion has been demonstrated for many commonly used anaesthetics such as diethyl ether, chloral hydrate, urethane and barbiturates (Lee & Thompson, 1967; Barret *et al*., 1978; Graffner *et al*., 1991; Bastaki *et al*., 1995). In contrast, there are also reports of stimulatory effects of pentobarbitone on acid secretion (Watanabe *et al*., 1987). It is not known whether the effect of anaesthetics is exerted on parietal cells directly or indirectly *via* depression (or excitation) of other cells/neurons involved in the acid secretory pathway. Histamine, released from the ECL cells in response to gastrin, is a powerful stimulus of acid secretion, and ECL-cell histamine is thought to play an important role in the fundamental control of the parietal cells (Kahlson *et al*., 1964; Håkanson & Sundler, 1991; Waldum *et al*., 1991; Black, 1993; Andersson *et al*., 1996). Evidence that gastrin acts by mobilizing gastrin histamine has come from studies of intact rats (Kahlson *et al*., 1964; Håkanson & Sundler, 1991; Chen *et al*., 1994; Kitano *et al*., 2000) or vascularly perfused, isolated rat stomachs (Sandvik *et al*., 1987; Waldum *et al*., 1991), from studies in which histamine has been monitored in the portal vein (Gerber & Payne, 1992; Aurang *et al*., 1997) and from studies of isolated ECL cells (Prinz *et al*., 1993; Lindström *et al*., 1997). Many of these experiments have been carried out on anaesthetized animals. When this study was initiated it was not known whether anaesthesia would affect the ability of the ECL cells to secrete histamine and their ability to respond to gastrin with histamine mobilization. Hence, it could not be excluded that the well-known impairment of acid secretion during anaesthesia reflected a reduced ability of the ECL cells to secrete histamine. The present study compares the effect of several anaesthetic agents on basal and gastrin-stimulated ECL-cell histamine mobilization, using (1) rats fitted with a microdialysis probe in the submucosa of the acid-producing part of the stomach, and (2) isolated ECL cells in primary culture. Acid secretion in conscious and anaesthetized rats was studied in rats equipped with a chronic gastric fistula. The technique of *in vivo* microdialysis has been shown to be useful for monitoring the mobilization of histamine from the ECL cells in conscious rats (Kitano *et al*., 2000). General anaesthesia, induced by chloral hydrate, pentobarbitone, urethane or a mixture of fluanisone/fentanyl/midazolam (neurolept analgesia) was without effect on basal histamine release but reduced gastrin-induced histamine mobilization by 55–80% with chloral hydrate being the most effective. In contrast, both the basal acid output and the gastrin-evoked acid response were greatly reduced by the anaesthetic agents tested, suggesting that anaesthesia inhibits not only ECL cells, but also parietal cells. Chloral hydrate virtually abolished the acid response to gastrin, and pentobarbitone, urethane and the mixture of fluanisone/fentanyl/midazolam reduced the acid response by about 70%. There was a high degree of correlation between the suppressive effect of various anaesthetic drugs on the gastrin-induced histamine mobilization and the gastrin-induced acid response. Hence, while the basal acid output seems to be independent of ECL-cell histamine secretion, impaired ECL-cell histamine secretion may at least partly explain the suppressive effects of anaesthesia on gastrin-stimulated acid secretion. Since gastrin-evoked histamine mobilization from isolated ECL cells was dose-dependently inhibited by the anaesthetic agents used in this study (except urethane), we propose that the suppressive effect is exerted directly on the ECL cell. Kawakubo and co-workers (1999) argued the urethane suppresses gastrin-induced acid secretion by stimulating gastric somatostatin release (see also Yang *et al*., 1990), an observation which makes it possible to explain the fact that urethane impairs ECL-cell function *in vivo* but not *in vitro*. It must be realized, however, that also histamine-evoked acid secretion is suppressed by anaesthesia: urethane, pentobarbitone and chloral hydrate almost completely inhibited histamine-stimulated acid secretion (Lee & Thompson, 1967; Graffner *et al*., 1991; Bastaki *et al*., 1995). Clearly, therefore, anaesthetic agents are capable of inhibiting not only the ECL cells but also the parietal cells.

Despite numerous reports that demonstrate inhibitory effects of anaesthesia on acid output, anaesthetized and surgically manipulated rats are still widely used in studies of gastric acid secretion. The present results indicate that studies of acid secretion and ECL-cell histamine mobilization are seriously compromised by the use of anaesthetics, leading us to argue that ECL-cell histamine mobilization (and the physiology of gastric acid secretion) is best studied in conscious subjects.

This study was supported by grants from the Swedish MRC (04X-1007) and the A. Påhlsson Foundation.

References

ANDERSSON, K., CABERO, J.L., MATTSSON, H. & HÅKANSON, R. (1996). Gastric acid secretion after depletion in enterochromaffin-like cell histamine. *Scand. J. Gastroenterol.,* **31,** 24–30.

ANDERSSON, K., CHEN, D., HÅKANSON, R., MATTSSON, H. & SUNDLER, F. (1992). Enterochromaffin-like cells in the rat stomach: effect of α-fluoromethylhistidine-evoked histamine depletion. *Cell Tissue Res.,* **270,** 7–13.

AURANG, K., SPRAGGS, C.F., JORDAN, C. & LLOYD, K.C.K. (1997). Role of gastrin/CCK-B receptors in meal-stimulated acid secretion in rats. *Am. J. Physiol.,* **272,** G1243–G1248.

BARRET, A.M., RAVENTOS, J. & SIDDAL, R.A. (1978). Influence of some anaesthetics on pharmacologically stimulated gastric secretion in rats. *Br. J. Pharmacol.,* **28,** 51–63.

BASTAKI, S.M.A., WATON, G. & GARNER, A. (1995). Effects of anaesthetic agents on basal and histamine-stimulated acid secretion in the fistula rat. *Eur. J. Gastroenterol. Hepatol.,* **7,** 1199–1202.

BEL, A., LEVRAT, R., NESMOZ, J. & GIRARD, M. (1966). Nouvelle technique de fistule gastrique chronique chez le rat. *J. Med. Lyon,* Special issue October, 5–11.

BLACK, J. (1993). Reflections on the analytical pharmacology of histamine-H_2 receptor antagonists. *Gastroenterology,* **105,** 963–968.

CHEN, D., MONSTEIN, H.J., NYLANDER, A.G., ZHAO, C.M., SUNDLER, F. & HÅKANSON, R. (1994). Acid responses of rat stomach enterochromaffin-like cells to gastrin: secretory activation and adaptation. *Gastroenterology,* **107,** 18–27.

DARTSCH, C., SUNDLER, F. & PERSSON, L. (1999). Antisera against rat recombinant histidine decarboxylase: immunocytochemical studies in different species. *Histochem. J.,* **31,** 507–514.

GERBER, J.G. & PAYNE, N.A. (1992). The role of gastric secretagogues in regulating gastric histamine release *in vivo*. *Gastroenterology,* **102,** 403–408.

GRAFFNER, H., EKELUND, M. & HÅKANSON, R. (1991). Anaesthetic agents suppress basal and stimulated gastric acid secretion. *Scand. J. Gastroenterol.,* **26,** 1200 – 1204.

HÅKANSON, R., BÖTTCHER, G., EKBLAD, E., PANULA, P., SIMMONSSON, M., DOHLSTEN, M., HALLBERG, T. & SUNDLER, F. (1986). Histamine in endocrine cells in the stomach. A survey of several species using a panel of histamine antibodies. *Histochemistry,* **86,** 5 – 17.

HÅKANSON, R., CHEN, D. & SUNDLER, F. (1994). The ECL cells. In *Physiology of the Gastrointestinal Tract*. ed. Johnson, L.R. Vol. 2, 3rd edn. pp. 1171 – 1184. New York: Raven Press.

HÅKANSON, R., DING, X.-Q., NORLÉN, P. & LINDSTRÖM, E. (1999). CCK_2 receptor antagonists: pharmacological tools to study the gastrin-ECL cell-parietal cell axis. *Regul. Pept.,* **80,** 1 – 12.

HÅKANSON, R. & SUNDLER, F. (1991). Do histamine-storing cells in the gastric mucosa mediate the acid-stimulating action of gastrin? In *Histamine and Histamine Antagonists. Handbook of Experimental Pharmacology*. ed. Uvnäs, B. pp. 325 – 346. Berlin: Springer Verlag.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol. (Lond.),* **174,** 400 – 416.

KAWAKUBO, K., COY, D.H., WALSH, J.H. & TACHÉ, Y. (1999). Urethane-induced somatostatin inhibition of gastric acid: reversal by the somatostatin 2 receptor antagonist, PRL-2903. *Life Sci.,* **65,** 115 – 120.

KITAMURA, M., SUGAMOTO, S., KAWAUCHI, S., KETO, S. & TAKEUCHI, K. (1999). Modulation by endogenous nitric oxide of acid secretion induced by gastric distension in rats: Enhancement by nitric oxide synthase inhibitor. *J. Pharmacol. Exp. Ther.,* **291,** 181 – 187.

KITANO, M., NORLÉN, P. & HÅKANSON, R. (2000). Gastric submucosal microdialysis: A method to study gastrin- and food-evoked mobilization of ECL-cell histamine in conscious rats. *Regul. Pept.,* **86,** 113 – 123.

LEE, Y.H. & THOMPSON, J.H. (1967). Effect of anesthetic agents on maximal histamine-induced gastric secretion in Shay rats. *Am. J. Physiol.,* **213,** 1331 – 1334.

LINDSTRÖM, E., BJÖRKQVIST, M., BOKETOFT, Å., CHEN, D., ZHAO, C.M., KIMURA, K. & HÅKANSON, R. (1997). Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. *Regul. Pept.,* **71,** 73 – 86.

MÅRVIK, R., SANDVIK, A.K. & WALDUM, H.L. (1995). Bioassay of gastrin using the isolated vascularly perfused rat stomach. A new, simplified and sensitive method. *Acta Physiol. Scand.,* **155,** 323 – 327.

PRINZ, C., KAJIMURA, M., SCOTT, D.R., MERCIER, F., HELANDER, H.F. & SACHS, G. (1993). Histamine release from rat enterochromaffinlike cells. *Gastroenterology,* **105,** 449 – 461.

SANDVIK, A.K., WALDUM, H.L., KLEVELAND, P.M. & SCHULTZE-SØGNEN, B. (1987). Gastrin produces an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated, vascularly perfused rat stomach. *Scand. J. Gastroenterol.,* **22,** 803 – 808.

SOLL, A.H., TOOMEY, M., CULP, D., SHANAHAN, F. & BEAVEN, M.A. (1988). Modulation of histamine release from canine fundic mucosal mast cells. *Am. J. Physiol.,* **254,** G40 – G48.

WALDUM, H.L., SANDVIK, A.K., BRENNA, E. & PETERSEN, H. (1991). The gastrin-histamine sequence in the regulation of gastric acid secretion. *Gut,* **32,** 698 – 700.

WATANABE, K., YANO, S. & LIN, W.C. (1987). Stimulatory effect of pentobarbitone and some anaesthetics on gastric acid secretion in the continuously perfused stomach in rats under urethane anaesthesia. *Jap. J. Pharmacol.,* **44,** 63 – 69.

YANG, H., WONG, H., WU, V., WALSH, J.H. & TACHÉ, Y. (1990). Somatostatin monoclonal antibody immunoneutralization increases gastrin and gastric acid secretion in urethane anesthetized rats. *Gastroenterology,* **99,** 659 – 665.

(Received November 19, 1999
Revised February 15, 2000
Accepted March 6, 2000)