ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

VETERINARIA 21

Acid Secretion and Gastrin Parameters after Inhibition of Acid Secretion and Interference with Sex Hormones in Rats

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Studies of acid secretion and gastrin parameters after inhibition of acid secretion and interference with sex hormones in rats

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Akademisk avhandling som för vinnande av veterinärmedicine doktorsexamen kommer att offentligen försvaras i Ettans föreläsningssal, Klinikcentrum, Ultuna, fredagen den 12 september 1997, kl. 13.15

Abstract

Ulcer disease is today treated with drugs that profoundly and with long duration inhibit the acid secretion and such treatment is often carried out for many years. This treatment may mean a risk for the patients.

In this thesis the effects of the H^+/K^+-ATPase inhibitor omeprazole and the H_2-receptor antagonist ranitidine are studied on acid secretion and some gastrin parameters. Both drugs inhibit basal acid secretion to a similar extent and omeprazole reduces stimulated secretion better than ranitidine. The treatment does not cause any tachyphylaxis or increased maximal secretion, but increased sensitivity to stimulation for omeprazole was seen. The total omeprazole dose can be reduced if it is given twice per day.

Fasting gradually lowers the plasma gastrin concentration as well as prolonged time between the last omeprazole dose and blood sampling. Tissue gastrin concentrations are relatively stable under these conditions.

Treatment with omeprazole and ranitidine for 28 days with doses that produce a similar 24 hour inhibition of the acid secretion increases the gastric gastrin content. Omeprazole raises the gastrin content already after 3 days and the final increase is much higher than for ranitidine. After a recovery period for 28 days, gastric concentrations are normalized.

Normal male rats secrete more acid than females in response to maximal stimulation and such daytime secretion is larger than at night. Gonadectomy reduces the acid output in males but not in females. Estradiol treatment does not influence basal acid secretion but testosterone lowers the basal acid output in females but not in males. In both sexes, estradiol and testosterone lower the stimulated daytime secretion but raise the secretion at night. Both hormones inhibits the stimulation of parietal cells in vitro.

Gonadectomy lowers the number of gastrin cells and the plasma gastrin concentration in both sexes. Treatment with testosterone lowers the number of gastrin cells and the plasma gastrin concentration. Estradiol also reduces the cell number but increases the plasma gastrin concentration.

Distribution: Swedish University of Agricultural Sciences, Uppsala 1997

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ISSN 1401-6257
ISBN 91 576 5412 3

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 1997

Acta Universitatis Agriculturae Sueciae
Veterinaria 21

ISSN 1401-6257
ISBN 91-576-5412-3

Tryck: SLU Service/Repro, Uppsala 1997

Life is a wonderful thing
one can be amazed of its diversity
one can be learned from its generalities

S.J. Gould

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LIST OF PUBLICATIONS

The thesis is based on the following papers, which will be referred to by their Roman numerals I-IV.

I. Rein Seensalu, Kinfe Girma, Bengt Romell and Göran Nilsson.
Time course of inhibition of gastric acid secretion in gastric fistula rats by omeprazole and ranitidine. Eur J Pharmacol 180:145-152, 1990.

II. Rein Seensalu, Kinfe Girma, Bengt Romell and Göran Nilsson.
Effects of omeprazole and ranitidine on plasma gastrin concentration and stomach gastrin content in rats. Upsala J Med Sci 97:157-167, 1992.

III. Kinfe Girma, Izabella Janczewska, Bengt Romell, Rein Seensalu, Andreas Sandin, Erik Wilander and Göran Nilsson.
Twenty-four-hour basal and repetitive pentagastrin-stimulated gastric acid secretion in normal and sham-operated rats and in rats after gonadectomy or treatment with estradiol or testosterone. Scand J Gastroenterol 32:669-675, 1997.

IV. Kinfe Girma, Izabella Janczewska, Bengt Romell, Rein Seensalu, Andreas Sandin, Erik Wilander and Göran Nilsson.
Repetitive determinations of plasma gastrin concentrations and number of gastrin cells in normal rats and following neonatal gonadectomy or treatment with estradiol or testosterone. Submitted for publication.

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CONTENTS

GENERAL BACKGROUND

Most pharmacological ulcer treatment has been carried out by drugs that exert their effects by reducing the gastric acid output. For many years, anticholinergic substances interfering with the acetylcholine receptors on the parietal cell membrane and antacids were the drugs of choice. Despite early strong evidences that histamine is involved in the activation of the parietal cell (Popielski 1920), no histamine antagonists producing a significant reduction of the acid output were available until 1972, when the first H_2-receptor antagonist was developed (Black et al. 1973). During the seventies and eighties the three H_2-antagonists cimetidine (Brimblecombe et al. 1975), ranitidine (Bradshaw et al. 1979; Brittain and Daly 1981; Daly, Humphray and Stables 1981) and famotidine (Takagi, Takeda and Maeno 1982) became the most commonly used drugs for treatment of ulcer disease.

The H_2-receptor antagonists interfere with the H_2-receptors on the cell membrane of the parietal cells and suppress both basal and stimulated acid secretion. During the eighties, a new drug, acting according to a different and so far not applied principle, became a new important tool for the treatment of ulcer disease (Larsson et al. 1983; Wallmark et al. 1983). This drug, omeprazole, acts intracellularly by inhibiting the proton pump within the parietal cell, where it binds irreversibly to the enzyme H^+/K^+-ATPase. Omeprazole produces a profound inhibition of the acid output and has a longer duration than the H_2-receptor antagonists (Larsson et al. 1983; Larsson, Mattsson and Carlsson 1988; Lind et al. 1983; Sharma et al. 1984). Pharmacological treatment of ulcer disease was earlier given during relatively short periods (4-8 weeks) after which time the disease mostly recurred. From the beginning of the eighties, it became common to continue the pharmacological treatment for prophylactic purposes when the ulcers were healed. It was even suggested that patients with duodenal ulcers and frequent recurrencies should be given a life long prophylactic treatment with acid inhibitory drugs (Wormsley 1982). The finding that a bacteria, Helicobacter pylori (Lee 1991; Peterson 1991), may play a role in ulcer disease and the use of antibiotic treatment in ulcer patients (Solcia et al 1992) has prevented recurrencies in many ulcer patients and therefore reduced the need of long term pharmacological treatment. However, the antibiotic treatment is not successful to 100 %, many patients are suffering from esophageal reflux

and some from gastrin secreting tumours. A lot of patients are therefore still given long-term treatment with acid inhibitory drugs.

There are several reasons to assume that profound suppression of the acid secretion for long periods of time may be harmful for the patients. A number of risks following long-term reduction of the gastric acid secretion can be identified.

The inhibition of the acid output decreases the acidity within the gastric antrum. When the antral pH increases to about 3, more gastrin will be released from the antrum and the plasma gastrin concentration increases (Woodward et al. 1954; Nilsson et al. 1972; Becker, Reeder and Thompson 1973a). Neutralization of the gastric content during longer periods of time will also raise the gastrin tissue content within the antrum and increase its number of gastrin cells (Lehy et al.1975; Alumets et al. 1979; Kaduk and Häuser 1980; Allen et al. 1986). Released gastrin not only contributes to the activation of the parietal cells causing increased acid secretion. Gastrin also exerts a trophic effect on the gastric mucosa (Crean, Marshall and Rumsey 1969; Johnson, Aures and Håkanson 1969; Witzel et al. 1977; Mazzacca et al. 1978; Håkanson et al. 1986). The capacity of the gastric mucosa to secrete acid may then increase. In rats, increased acid secretion has been found following treatment with the H_2-receptor antagonist metiamide (Witzel et al. 1977) and the H^+/K^+-ATPase inhibitor omeprazole (Larsson et al. 1988). Hypersecretion of acid in humans has been demonstrated following the use of H_2-receptor antagonists (Sewing et al.1978; Frislid, Aadland and Berstad 1986; Jones et al. 1988; Fullarton et al. 1989).

The trophic effect by gastrin involves not only the parietal cells but also endocrine cells of the acid producing mucosa. In particular, the effects on the enterochromaffin-like (ECL) cells have been studied. A number of experimental procedures or clinical conditions may induce ECL-cell hyperplasia. Thus, such hyperplasia has been seen in animals following surgical procedures (Håkanson et al. 1976; Alumets et al. 1979; Tielemans et al. 1990) or pharmacological (Larsson et al. 1986; Axelson et al. 1988; Ryberg et al. 1989; Tielemans et al. 1989) treatment in animals that increase the plasma gastrin concentration. In humans, it has been found that hypergastrinemia in association with achlorhydria caused

by chronic atrophic gastritis or pernicious anemia (Rubin 1969; Bordi, Costa and Missale 1975; Borch 1985; Cattan et al. 1989) or gastrin producing tumours (Bordi et al. 1974; Lehy et al.1989; Solcia, Capella and Vassallo 1970; Solcia et al. 1975) raise the number of ECL-cells in the acid producing portion of the stomach. Treatment of rats for 2 years with various doses (5-400 μmol/kg/day) of omeprazole has produced a dose related number of carcinoid tumours in female rats. Surprisingly, the tumours in male rats were much less common and only found after treatment with the highest omeprazole concentrations (Ekman et al. 1985; Information från Socialstyrelsens Läkemedelsavdelning 1988). No satisfactory explanation has been given to this difference. Long term treatment of female rats with very high doses of ranitidine (2 g/kg/day) has also caused carcinoid tumours (Havu et al. 1990).

There are also reports of gastric tumours that have been found in toxicological investigations of some other acid inhibitory drugs. Thus, neoplasms similar to those developed by omeprazole and ranitidine have been demonstrated in both mice and rats after treatment with the H_2-receptor antagonists loxtidine (Poynter et al. 1985), BL-6341 (Hirth et al. 1988), ICI 162,846 (Streett, Robertson and Crissman 1988) and lupitidine (Harleman et al. 1987). Also other gastric tumours have been observed after treatment with acid inhibitory drugs. Thus, lupitidine has been reported to cause squamous cell carcinoma in the rumenal mucosa of the rats (Betton and Salmon 1984), and tiotidine has given adenocarcinomas of the antral mucosa (Streett, Cimprich and Robertson 1984). Whether the mentioned tumours can be attributed to increases in plasma gastrin concentrations or whether other factors contribute to the development of the tumours cannot be decided so far.

In several animal studies, gastrin seems to exert a promoting effect on tumour growth (Tahara and Haizuka 1975; McGregor et al. 1982; Tahara et al. 1982). In some studies, colonic carcinomas in mice and rats have been exposed to exogenous gastrin (Svet-Moldavsky 1980; Winsett et al. 1986; Sumiyoshi et al. 1984). Gastrin has also been demonstrated to influence human colon cancer (Sirinek, Levine and Moyer 1985) and gastric carcinoma cell lines (Ochiai, Yasui and Tahara 1985). Also, gastrin receptors have been demonstrated on tumour cells from colon cancer (Rac-Venter et al. 1981) and from scirrous gastric cancer in patients (Kumamoto 1988; Weinstock and Baldwin 1988). It is not known whether

elevated concentrations of gastrin in plasma following acid suppressing treatment may accelerate the growth of tumours in man.

In still other studies, it has been found that profound and long lasting inhibition of the acid output causes an increased number of gastric bacteria in animals (Alam, Saporoschetz and Epstein 1971) and humans (Gray and Shiner 1967; Drasar, Shiner and McLeod 1969). Some of these bacteria are able to convert salivary and dietary nitrate to nitrite. In the presence of a high intragastric pH, nitrite will form N-nitroso derivates in the stomach (Hawksworth and Hill 1971; Reed et al 1981) and such compounds are believed to participate in the development of gastric tumours (Correa et al. 1975; Ruddell et al. 1978; Lijinsky 1979; Tahara et al. 1981).

Previous studies in which the role of sex hormones on acid secretion have been investigated have given conflicting results. A study in man failed to show inhibition (Parbhoo and Johnston 1966). Other investigations in man (Sakaguchi et al. 1991) and studies in cats (Ojha and Wood 1950), guinea pigs (Katz, Herr and Kovacs 1969), rabbits (Maitrya, Gahlot and Maitrya 1979) and rats (Amure and Omole 1970; Maitrya, Gahlot and Maitrya 1979; Piyachaturawat et al. 1983) have shown a reduced acid output. Results from dogs (Landor and Wild 1970) and rats (Omole 1972) have indicated lowered acid secretion during estrus and studies in rats have shown reduced acid secretion during pregnancy (Lozzio et al. 1961).

Administration of the male sex hormone testosterone has increased acid secretion in dogs (Eisenberg, Owens and Woodward 1965) and in female but not in male rats and in rabbits (Maitrya, Ghalot and Maitrya 1979). In male rats, testosterone instead inhibited acid secretion (Maitrya, Ghalot and Maitrya 1979). In experiments, where the sex glands were surgically removed, Olowo-Okurun (1975) did not find any considerable effects on basal acid secretion. Amure and Omole (1970), on the other hand, observed reduced histamine stimulated acid secretion in both sexes.

The related results have mostly been obtained from experiments that have been carried out during a few (2-6) hours. In most cases the animals have been anaesthetized with barbiturates or urethane. In rats, the pylorus ligation technique, elaborated by Shay and cow. (1954) or the perfusion

technique according to Ghosh and Schild (1958) have been used. In most studies changes in acid secretion have been estimated qualitatively by pH-determinations of the gastric content and not quantitatively by measuring the secreted volume and the acidity of the acid output by titration. In addition, the periods during which sex hormones have been administered vary considerably between the studies.

The mentioned results suggest that sex hormones may exert an influence on the gastric acid secretion, but due to the related experimental conditions, it is difficult to estimate the physiological significance of the results reported so far. Recently, another indication has been obtained that sex hormones may influence the acid secretion. Thus, it has been demonstrated that testosterone may be synthetized in the parietal cells (Le-Goascogne et al. 1995).

The great difference between females and males in gastric carcinoid tumour rate, as related above, together with the insignificant amount of information available considering the role of sex hormones in influencing the exocrine and endocrine secretions of the stomach, motivate further studies on this subject.

REGULATION OF GASTRIC ACID SECRETION

The stomach of the rat is divided into three parts. The upper non-glandular area has a squamous epithelium and no acid is secreted there. In the middle secretory area (the corpus) acid, pepsin, mucous and bicarbonate are produced and secreted and in the distal endocrine part, the antrum, the hormone gastrin is synthetized and secreted. Also mucous and bicarbonate are secreted there in order to protect the mucosa.

The acid secreting portion occupies the major part of the stomach. The parietal cell from which the acid is secreted is rich in mitochondriae. From them the energy is generated that is required for the secretion of hydrogen ions. The parietal cell also contains tubulovesicular and canalicular structures (Ito 1987; Helander 1988). An enzyme, the H^+/K^+-ATPase takes part in the exchange of hydrogen and potassium ions across the vesicular membrane of the acid secreting cell. Microscopically, a resting and a secretory state can be distinguished in the parietal cell. Very soon after the start of a stimulation the morphological structure of the

cell will change and the secretory area becomes greatly increased (Helander and Hirschowitz 1972; Black, Forte and Forte 1980; Helander and Sundell 1984; Helander, Leth and Olbe 1986).

Three main factors are considered to take part in the activation of the parietal cell. At a meal, acetylcholine is released from postsynaptic fibers of the vagal nerves and acts on muscarinic receptors located on the outside of the parietal cell membrane (Berglindh 1977; Soll 1978; Ecknauer et al. 1981). The vagal stimulation also liberates gastrin from the antral portion of the stomach (Uvnäs 1942; Nilsson et al. 1972), which in turn may act stimulating on the parietal cells, since gastrin receptors have been demonstrated on these cells (Soll et al. 1984). The released gastrin in particular acts on the ECL-cells which in the rat are located in close connection with the parietal cells (Thunberg 1967; Ehinger et al. 1968; Håkanson et al. 1976; Håkanson et al. 1986). The ECL-cells are most likely also directly stimulated by vagal excitation (Bergqvist et al. 1980; Nylander, Bergqvist and Öbrink 1985). Histamine is formed in the ECL-cells in the rat, when the enzyme histidine decarboxylase is activated by gastrin (Håkanson et al. 1974). The histamine is then secreted from the ECL-cells and acts in a paracrine way on H_2-receptors on the parietal cells (Berglindh 1977; Soll 1978).

Following activation of the receptors on the parietal cell membrane, the excitation will be forwarded intracellularly by different messenger systems. Cholinergic and gastrin stimulation are supposed to be mediated by a calcium dependent mechanism that involves cytosolic calcium (Soll 1981). H_2-receptor stimulation will activate the adenylate cyclase system present in the cell membrane and cyclic AMP is formed (Soll and Wollin 1979). Activation of the H^+/K^+-ATPase enzyme is considered to be the last step in the chain of intra-cellular reactions and will result in the secretion of hydrogen ions.

Gastrin and cholinergic excitation also influence the somatostatin (D) cells in the acid secreting mucosa. Gastrin increases (Soll et al 1984; Soll et al. 1984) and cholinergic excitation (Yamada et al. 1984) inhibits the secretion of somatostatin from the D cells. Somatostatin acts on the parietal cells by inhibiting the secretion from these cells (Makhlouf and Schubert 1990).

Another factor contributing to the control of the acid secretion is the intra-antral pH (Woodward et al. 1954; Nilsson et al. 1972; Becker, Reeder and Thompson 1973a). When this pH is reduced below 3, gastrin release to the blood is diminished and the stimulation of the parietal cell is decreased. A pH-sensitive inhibitory mechanism also seems to operate in the duodenal bulb. When the intra-bulbar pH is reduced below 5, a humoral factor is considered to be released resulting in inhibition of the acid secretion (Nilsson 1980). Inhibition is also initiated when digestion products of fat act on the upper part of the small intestine (Nilsson 1979).

GASTRIN

Historical aspects and chemistry

Edkins (1906) was the first to claim that a humoral factor extracted from the antral portion of the stomach stimulated the gastric acid secretion. The principle was named gastrin, was finally isolated in 1964 and found to be an heptadecapeptide (G17) appearing in one sulphated and one non-sulphated form (Gregory and Tracy 1964). Later, a larger molecular form, (G34) was discovered (Yalow and Berson 1970) and isolated (Gregory and Tracy 1972). Gastrin forms that are minor than the hepta-decapeptide have also been found and isolated (Gregory and Tracy 1974). Differences between species in amino acid sequences have been noted and the distribution of gastrins of molecular size and the degree of sulphation have also been shown to vary between species. For a review see Johnsen and Rehfeld 1993. The C-terminal portion of the molecule is essential for the biological activity and the C-terminal tetrapeptide is the shortest sequence that exert all the biological effects of gastrin (Tracy and Gregory 1964). No difference in biological effect of the G17 and G34 peptides has been found (Eysselein et al. 1984). The half life in plasma for G 17 varies between species and has been shown to be 3 min in the dog (Straus and Yalow 1974) and 6 min in man (Eysselein et al. 1984). Corresponding figures for G34 are 9-15 and 24-36 min. Gastrin is essentially metabolized in the kidneys (Davidson, Springberg and Falkinburg 1973) and to some extent also by the small intestine (Becker, Reeder and Thompson 1973b).

Factors regulating gastrin secretion

The secretion is influenced by a number of stimulatory and inhibitory factors.

Stimulatory factors

Nervous influences

Vagal excitation induced by for example electrical vagal stimulation (Uvnäs 1942; Uvnäs, Uvnäs-Wallensten and Nilsson 1975) or sham feeding (Nilsson et al. 1972) will release gastrin. The vagal excitation is supposed to be mediated by cholinergic fibers releasing acetylcholine (Csendes, Walsh and Grossman 1972; Nilsson et al. 1972) and fibers containing the gastrin releasing peptide (GRP), which is the mammalian form of bombesin (McDonald et al. 1978; Ekblad et al. 1985). The intrinsic nervous system of the stomach also seems to contribute to the stimulation. Thus, distention of the acid secreting area of the stomach will induce gastrin release. This mechanism has been named the oxynto-pyloric reflex (Debas, Walsh and Grossman 1975). There are also indications that the adrenergic nervous system is involved in the stimulation, inasmuch as ß-adrenergic drugs release gastrin (Lundell, Svensson and Nilsson 1976).

Luminal influences

Experiments on dogs with isolated antral pouches indicate that amino acids and small peptide fragments cause gastrin release (Elwin 1974; Lichtenberger 1982; Strunz, Walsh and Grossman 1978). The mechanism by which such stimulation is mediated is not known.

Inhibitory factors

Gastrin release under stimulated conditions is reduced when the luminal pH in the antrum is reduced below 3. The mechanism behind this inhibition is not fully understood. Possibly, the inhibition is mediated by a paracrine mechanism. Microscopical studies have shown that somatostatin producing cells are present in the antral mucosa and that cytoplasmic processes from these cells are in contact with the gastrin cells (Alumets et al. 1979; Larsson et al. 1979). Somatostatin inhibits the release of gastrin

and is released during acidification of the antral lumen (Schusdziarra et al. 1978; Schusdziarra et al. 1979, Uvnäs-Wallensten et al. 1980; Koop et al. 1988).

It has also been suggested that cholinergic influences may act inhibiting on gastrin release (Walsh, Yalow and Berson 1971; Feldman et al. 1979).

Trophic effects of gastrin

A number of studies have shown that gastrin exerts a trophic effect on the oxyntic mucosa. Long term administration of pentagastrin increases the volume of the oxyntic mucosa and the number of the parietal cells (Crean, Marshall and Rumsey 1969). Administered gastrin also induce protein synthesis (Johnson, Aures and Håkanson 1969; Johnson, Aures and Yuen 1969) and stimulates the proliferation of endocrine cells in the corpus region of the stomach (Ryberg et al. 1989). Experimental manipulations in order to raise the endogenous release of gastrin also increase the mucosal height and the number of parietal and endocrine cells in the acid secreting portion of the stomach (Håkanson et al. 1986; Håkanson et al. 1988). Oppositely, antrectomy causes atrophy of the oxyntic mucosa (Martin, Macleod and Sircus 1970) and the gastric mucosal content of RNA and DNA will decrease (Johnson and Chandler 1973). This latter change is prevented if the antrectomized animals are treated with pentagastrin.

GASTRIN AND ACID SECRETION DURING A MEAL AND FASTING

Stimulated conditions

During a meal the sensory organs mediating sight, smell and taste will become activated and afferent nervous impulses will reach the brain and its vagal center. Efferent vagal impulses will arise and reach the antral and the acid secreting mucosae (Nilsson et al. 1972; Feldman and Richardson 1986). The plasma gastrin concentration will increase immediately and is further elevated when the food enters the stomach (Nilsson et al. 1972). Distention (Debas, Walsh and Grossman 1975) and chemical (Elwin 1974; Blair et al. 1975; Strunz, Walsh and Grossman 1978) stimulation by food components contribute to the intra-gastric

stimulation. Although gastrin release is immediate, there is a delay in the onset of acid secretion and the maximal acid output is reached first after 30-45 min (Nilsson et al. 1972). This delay is most likely due to a mechanism within the parietal cells, which is supposed to prevent acid to leave the cells until a certain intracanalicular acidity has been reached (Nilsson et al 1993). Due to the elevated acid secretion, the gastric content becomes increasingly acidified, which will contribute to a reduction of the plasma gastrin concentration (Woodward et al. 1954; Nilsson et al. 1972; Becker, Reeder and Thompson 1973a). The emptying of the acid gastric content into the duodenal bulb will activate a postulated pH-sensitive inhibitory mechanism there that is supposed to contribute to the arrest of acid secretion after a meal and to suppress acid secretions arising between meals (Nilsson 1980).

There are several studies indicating that also the small intestine is involved in the control of acid secretion. With the exception for the duodenal bulb inhibitory mechanism, this field is less well investigated. It is, however, an established fact that fatty acids in contact with the mucosa of the upper small intestine cause inhibition of the gastric acid secretion (Nilsson 1979).

Basal conditions

Both gastrin and acid are secreted during basal or fasting conditions. The mechanisms controlling these secretions are not well understood and may vary between species. Whereas the intra-antral pH plays a role in controlling the gastrin release during stimulation, this mechanism does not seem to be operating during fasting (Debas et al. 1974). When fasting is prolonged, as during starving, there is a reduction in the plasma and antral gastrin concentrations (Lichtenberger, Lechago and Johnson 1975; Lichtenberger 1982) and in the number of gastrin cells (Bertrand and Willems 1980; Schwarting et al. 1986), indicating that the presence of food in the stomach exerts a stimulatory effect on the gastrin cells, resulting in both proliferation of the cells and an increase of their secretion.

GASTRIC ENDOCRINE CELLS

Endocrine cells of two types are present in the gastric mucosa. In the antrum gastrin (G), somatostatin (D) and enterochromaffin (EC) cells are found. These cells are "open" and reach the lumen of the gastric gland with an apical process. It is assumed that these cells may be directly influenced by factors present in the antral lumen. The endocrine cells (ECL cells, A-like cells and D-cells) in the oxyntic gland area, on the other hand, lack this contact with the lumen and therefore are referred to as "closed" (Håkanson, Ekelund and Sundler 1984).

Gastrin cells

The antral mucosa is the main source of gastrin, although certain amounts of gastrin also may be found in the small intestine with decreasing concentrations in distal direction (Nilsson, Yalow and Berson 1973). In the antrum, there are certain species variations concerning the location of the gastrin cells within the glands. In rats, mice and rabbits, but not in man, the gastrin producing cells are most frequent in the basal portion of the glands (Solcia et al. 1975). Ultrastructural studies indicate that an acute elevation of the antral pH will activate the G-cell and following prolonged elevation of the pH, induced by different means, the G-cells are enlarged, the number of secretory granules are reduced, the proportion of electron dense granules is increased and there is an increase in the endoplasmatic reticulum and the Golgi area (Alumets et al. 1980; Håkanson et al. 1982). After a profound and long standing inhibition of the acid secretion in rats, features of G-cell hyperplasia such as clustering of the G-cells and "microadenomas" will appear in the antral mucosa (Håkanson, Ekelund and Sundler 1984). If the intra-antral pH is raised following antral exclusion or antrocolic transposition in rats, no G-cell proliferation is found (Alumets et al. 1980). Oppositely, starvation in rats reduces the number of gastrin cells (Lichtenberger, Lechago and Johnson 1975; Bertrand and Willems 1980; Schwarting et al. 1986). Long standing pharmacological reduction of the intra-gastric acidity by ant-acids (Kaduk and Häuser 1980; Koop et al. 1988), H_2-antagonists (Del Tacca et al. 1987) or substituted benzimidazoles (Allen et al. 1986; Creutzfeld et al. 1986; Koop et al. 1987; Ryberg et al. 1989) will produce a considerable increase in the number of antral G-cells.

Somatostatin cells (D-cells)

D-cells producing and secreting somatostatin are found in tissues from different parts of the gastrointestinal tract. Such cells are provided with long slender cytoplasmatic processes which often have club-like endings in connection with the cells they are influencing (Alumets et al. 1979; Larsson et al. 1979). D-cells having this kind of processes have been found close to the G-cells in the pyloric antrum. This is considered as the morphological basis for the paracrine inhibitory effect that somatostatin exerts on the gastrin cells. D-cells are supposed to exert a similar effect on the parietal cells in the corpus mucosa (Håkanson, Ekelund and Sundler 1984).

Enterochromaffin cells (EC-cells)

The EC-cell distribution within the gastric mucosa varies considerably between one species and another. The cells are histochemically detected due to their content of 5-hydroxytryptamine (5-HT). In rats, mice and hamster, most EC-cells are found in the antral mucosa (Håkanson et al. 1970). The physiological role of these antral cells or how their functions are regulated is not known. Release of 5-HT by vagal stimulation has been demonstrated from the intestine (Ahlman 1976). In one study, treatment of rats with omeprazole has been shown to lower the number of EC-cells (Allen et al. 1986), whereas no effects were noted in another study in which the rats were treated with omeprazole or ranitidine (Ryberg et al. 1989).

Enterochromaffin-like cells (ECL-cells)

The ECL-cells constitute 60-70 % of the endocrine cells in the acid producing area of the rat stomach (Håkanson et al. 1976). Little is known for most species about the physiological functions of these cells and how these functions are controlled. In the rat, the cells produce, store and release histamine (Håkanson and Owman 1967; Håkanson and Owman 1969) that is considered to be one of the most important factors for the activation of the closely located parietal cells. At stimulation of the ECL-cells, the enzyme histidine decarboxylase (HD) within the ECL-cells is activated, histamine is formed from histidine and then released. The ECL-cell concentration of (HD) reflects the activity of the ECL-cells

(Håkanson et al. 1974; Ryberg et al. 1989). A correlation has been indicated between the plasma gastrin concentration and the intracellular HD-activity. Thus, lowering of the plasma gastrin concentration by surgical removal of the antrum will reduce the number and size of the ECL-cells (Håkanson et al. 1976; Larsson et al. 1986; Tielemans et al. 1990). If, on the other hand, the gastric acid secretion is profoundly reduced by drugs (Larsson et al. 1986; Ryberg et al. 1989; Tielemans et al. 1989), or by surgical exclusion of the antrum (Alumets et al. 1979; Tielemans et al. 1990), hyperplasia of the ECL-cells will develope. Also other factors seem to be able to exert trophic influences on the ECL-cells. Thus, unilateral vagotomy in rats will cause a considerable loss of the mucosal height and of the number of ECL-cells in the denervated portion of the acid producing part of the stomach, indicating that vagal fibers contribute to the stimulation of the ECL-cells (Håkanson et al. 1984). Other studies have shown that the combined action of antrectomy and vagal denervation further reduce the density of the ECL-cells (Axelson et al. 1988). However, also other factors are supposed to exert an influence on the ECL-cells. Thus, there is a considerable increase in the number of ECL-cells, but no elevation of the plasma gastrin concentration, when rats are provided with a portacaval shunt (Reichle et al. 1974; Håkanson et al. 1976; Ekelund et al. 1985). The nature of this trophic activity remains to be investigated.

The South African rodent called Mastomys may develope ECL-cell tumours ("ECLomas" or carcinoid tumours) from the ECL-cells spontaneously (Snell and Stewart 1969; Modlin et al. 1988). ECL-cells in normal rats from other strains usually used in animal experiments, do not spontaneously undergo neoplastic transformations. However, following exposure to some acid inhibitory drugs, such as omeprazole and ranitidine, carcinoid tumours may develope also in such rats. Gastric carcinoid tumours have also been described in man in diseases that are associated with atrophic changes of the acid secreting mucosa and hypergastrinemia (Solcia et al. 1975; Johansson and Wilander 1982; Carney et al. 1983; Wilander, El-Salhy and Pitkanen 1984; Harvey et al. 1985; Borch 1985; Mignon et al. 1987; Goldfain et al. 1989).

A-like cells

Approximately 25 % of the endocrine cells in the acid secreting mucosa of the rat stomach are A-like cells (Håkanson et al. 1976). They have characteristics in common with the ECL-cells but do not contain histamine (Kubota et al. 1984). These cells are supposed to be peptide producing. However, so far the physiological function is not understood.

ACID INHIBITORY DRUGS

H_2-receptor antagonists

After that the H_1-receptor had been defined in 1966 (Ash and Schild), the H_2-receptor was soon described (Black et al. 1972) and the first antagonist to this receptor was presented (Black et al. 1973). Cimetidine, ranitidine and famotidine are today the most commonly used antagonists in clinical praxis. Ranitidine, that has been used in the present study, has a furan structure. It has been shown to inhibit basal acid secretion and secretions stimulated by pentagastrin, histamine, db cAMP or cholinergic excitation (Bradshaw et al. 1979; Brittain and Daly 1981; Daly, Humphray and Stables 1981). 30-60 % of the administered dose is excreted through the kidney and 40 % of the excreted ranitidine appears unchanged in the urine (Bell et al. 1980).

Omeprazole

H^+/K^+-ATPase is an enzyme that takes part in the exchange of hydrogen and potassium ions in the secretory canalicular membranes in the parietal cell and the enzyme is supposed to contribute to the final formation of acid. Omeprazole is chemically a substituted benzimidazole and functionally a specific inhibitor of the H^+/K^+-ATPase enzyme. Omeprazole is a lipid-permeable weak base that becomes protonated. The protonated form is unstable and becomes converted to a sulphenamide derivate that is the active component of the drug (Wallmark, Larsson and Humble 1985; Lindberg et al. 1986). It binds covalently to sulphydryl groups of the enzyme that then becomes irreversibly inactivated (Im, Blakeman and Davis 1985). The ED_{50} of omeprazole in rats is about 10 μmol/kg (Larsson et al. 1986). The biological availability of the drug is about 5 % in freely fed and 10 % in fasting rats. In man, the biological availability

is around 60 %, whether the patients are fasting or not (Information från Socialstyrelsens Läkemedelsavdelning 1988). 20-30 % of omeprazole is secreted as metabolites in the urine. The remaining fraction is recovered in the faeces within 72 hours after administration (Skånberg, Hoffmann and Regårdh 1986).

Following the administration for three days to rats of a high (400 μmol/kg/day) dose of omeprazole, acid secretion and H^+/K^+-ATPase activity have returned to normal levels 48 hours after the last dosing. Following a lower dose (40 μmol/kg/day), control levels are reached after 24-30 hours (Wallmark, Larsson and Humble 1985). Omeprazole causes a profound and long lasting inhibition of the acid secretion in several species and it is an effective suppressor of basal acid secretion (Larsson et al 1983; Larsson et al. 1988) and secretions stimulated by pentagastrin (Larsson et al. 1983; Konturek et al. 1984), histamine (Larsson et al. 1983; Konturek et al. 1984; Larsson, Mattsson and Carlsson 1988) and carbachol (Wallmark, Larsson and Humble 1985; Larsson et al 1988).

AIMS OF THE PRESENT STUDY

1. To investigate how different doses of the H_2-receptor antagonist ranitidine and the H^+/K^+-ATPase inhibitor omeprazole influence basal and stimulated acid secretion in conscious male rats following short- and long-term administration. Attempts were made to find doses of the two substances that produce a corresponding degree of acid inhibition. The possible development of tachyphylaxis during treatment or increases in acid secretion after the end of the treatment were also studied.

2. To study how gastrin concentrations in plasma and gastric tissue are influenced by the conditions under which samples are collected, how gastrin concentrations are influenced following 4 weeks of treatment with omeprazole and ranitidine and after another 4 weeks of recovery and to study the time course of changes in tissue gastrin concentrations after treatment with omeprazole for 1- 28 days.

3. To study how neonatal gonadectomy or long-term treatment of normal male and female rats with estradiol or testosterone influence basal and stimulated acid secretion.

4. To study how neonatal gonadectomy or long-term treatment of normal male and female rats with estradiol or testosterone influence the number of gastrin cells and the plasma gastrin concentration.

MATERIAL AND METHODS

Animals

Sprague Dawley rats of both sexes weighing 180-500 g were used. The animals were kept in plastic cages in rooms with climate control and were fed a standard laboratory diet (AB Ewos, Sweden). Between experiments the animals were allowed to eat and drink freely. Body weights were determined weekly.

Surgical procedures

Anaesthesia

After 16-18 hours of fasting, rats used for acid secretion experiments were anaesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg) and given a gastric cannula. Animals that were gonadectomized were operated soon after birth and were anaesthetized by cooling on ice (Hetta 1978). 7-9 weeks after birth these animals were also provided with a gastric cannula and then anaesthetized with pentobarbital. After all operations animals were kept warm until they had recovered from the anaesthesia.

Gastric cannula operations

During these operations, a stainless steel cannula was inserted into the glandular portion of the stomach. After the operation, the animals were given 10 ml of Ringer's glucose (ACO, Sweden) subcutaneously and were allowed to recover for three weeks before acid secretion experiments were started.

Surgical removal of the sex glands

In male rats, the orchidectomy was carried out within 24 hours after birth, whereas the female rats were ovariectomized after 5-6 days. In both male and female animals, incisions were closed by histoacryl blue. When found to be necessary, a light microscope was used during the ovariectomies.

Acid secretion tests

Before experimental series were started, the animals were trained for at least three weeks to stand in Bollman cages. When experiments were carried out, the animals were fasted for 18-20 hours but had free access to water. During fasting, the animals were kept in special cages in order to prevent coprophagy. Before the start of the experiments, the gastric cannula was opened for 30 min and rinsed to allow a free passage of gastric juice. During the experiments, basal acid secretion was collected for one hour. The acid inhibitory drugs or the vehicle in which they were dissolved were then given orally and to ensure a complete absorption of the administered substances, the first one hour portion of gastric juice was not collected until after two hours. Due to the purpose with the different secretory series, acid secretion was followed for various periods of times.

In stimulation experiments, acid secretion was activated with pentagastrin (Peptavlon, ICI, England; 500 μg/kg or 650 nmol/kg) or histamine dihydrochloride (E. Merck, AG, Darmstadt, Germany; 1.1 or 136 μmol/kg) administered by subcutaneous infusion. At collection of gastric juice, the volume was measured and the acidity determined by titration with 0.1 M NaOH to pH 7.0, using equipment for automatic endpoint titration (Radiometer, Copenhagen, Denmark). To avoid dehydration during experiments, losses of fluid were replaced by subcutaneous injections or infusions of saline or Ringer's glucose (I, II and III).

In vitro experiments

Preparation procedures

Freely fed Sprague-Dawley male and female rats weighing 130-150 g were killed by cervical dislocation. The stomachs were taken out, rinsed and transformed into everted sacs and then taken through a series of enzyme treatments resulting in a suspension of cells having a viability of more than 90 % and containing about 20 % of parietal cells. The crude suspension of isolated cells was then fractioned by counterflow centrifugation using a Beckman elutriator rotor (JE 6B) in a J2-21 M/E centrifuge (Beckman Instruments, USA). A fraction containing an average portion of 75±3 % of parietal cells and a cell viability higher than 90 % was then collected. After concluded preincubation for 2 hours the enriched cell suspension was used for experiments. Further details of the preparation procedure is given in paper III.

Aminopyrine (AP) accumulation

In experiments the cells were incubated with histamine (10^{-4}M) for activation of the cells and with various (10^{-9}-10^{-4} M) doses of estradiol or testosterone. 0.1 mM of IMX, a methylxanthine derivate was always present in order to inhibit the parietal cell phosphodiesterase. A 400μl amount of the isolated rat parietal cell suspension ($5x10^6$ cells/ml) was incubated at 37°C in flat-bottomed plastic vials containing 1μM AP and the reagents in a shaking water bath. After 40 min, 200μl of the suspension was layered over 1 ml of a buffer medium and spun down in an Eppendorf table centrifuge at 12000 rpm for 10 sec. The pellet was then dissolved in 400 μl of 1 mM NaOH and 3 ml of scintillation fluid was added. The radioactivity was determined in a liquid scintillation counter.

Radioimmunoassay of gastrin

In experiments belonging to paper I and II, blood for determination of the gastrin concentration was collected from the jugular and carotid vessels or by exsanguination via the abdominal portion of the aorta when animals were killed. Various periods of fasting or freely fed conditions preceded these experiments.

In experiments in paper IV, the blood was collected from the tail in conscious animals every fourth hour during a 24 hour period when animals were freely fed.

Collection of gastric tissue was started after that the animals were bled. The stomach was taken out, tissues along the curvatures were removed, the stomach was opened along the major curvature, rinsed with saline, weighed and then frozen on dry ice and stored at -20°C. The frozen stomachs were later homogenized and boiled in water (w/v 1:10) for 10 min. Following cooling to room temperature the extract was filtered through gauze, the volume of the filtrate was adjusted to 25 ml and then stored at -20°C until the gastrin content was determined by radioimmunoassay (Nilsson 1975) using gastrin antibodies generously supplied by professor Jens Rehfeld, Copenhagen, Denmark and isotope labelled gastrin produced by Milab, Malmö, Sweden. All gastrin determinations were performed in duplicates and extracts were in addition measured in serial dilutions. The inter- and intra-assay variations were 8 and 12 %, respectively.

Immunohistochemistry

Antral tissue specimens (2-4 mm strips) were rapidly fixed in a 4 % formaldehyde solution and then embedded in paraffin. At least 4-6 sections were cut from each block. Gastrin cells were stained using the avidin-biotin complex technique (Wood and Warnke 1981), with a primary polyclonal antibody at a dilution of 1:1000. Normal human antral mucosa was used as a positive control of the immunoreaction and negative controls were obtained by omission of the primary antiserum.

The gastrin cells were counted using a reticle of 1 cm^2 divided into 100 small squares. The gauze was kept at the eye piece and sections were viewed at a magnification of x 500. The number of gastrin cells in each rat was calculated by point sampling technique from the average of three randomly selected visual fields. Only gastrin cells that were located at the corners of each square were counted and expressed as the number of cells/count. The entire mucosal thickness and the nuclei of the cells had to be visible to be accepted for counting.

Pharmacological treatment

Omeprazole (AB Hässle, Sweden) and ranitidine (Glaxo Operations UK Ltd, England) were dispersed or dissolved in 0.5 % Methocel, (hydroxypropylmethylcellulose) Dow Corning Corp, Midland, USA). pH of the suspensions were adjusted to 9.0. The suspensions were kept in refrigerator and renewed every 6th day (omeprazole) or twice weekly (ranitidine) to assure stability. The volumes that were administered orally by gavage were adjusted to the weight of the rats and varied between 2.7-3.3 ml. Omeprazole was in most experiments given twice and ranitidine four times daily.

When rats were given sex hormones, they were injected intramuscularly once per month with slow release preparations of estradiol (Estradurin®, Kabi-Pharmacia, Sweden; 1.8 mg/kg/month) or testosterone (Testosteron-depot®, Schering AG, Germany; 15 mg/kg/month). The first injection was given when the animals were 3 months old and the last at 6 months of age (III-IV).

In the in vitro experiments (III), female and male rat parietal cells were incubated with various (10^{-9}-10^{-4}M) doses of estradiol or testosterone for 40 min.

Statistical anlyses and treatment of results

In comparing acid secretion in controls and rats treated with omeprazole or ranitidine (paper I), the non-parametric Wilcoxon matched-pairs signed-rank test was applied.

In paper II, regression analyses were applied in order to statistically evaluate differences in plasma and tissue gastrin concentrations in animals killed on different occasions and following different experimental procedures.

When tissue gastrin concentrations were compared with controls after 28 days of treatment and following 28 days of recovery, analysis of variance and Bonferroni's test were carried out for multiple comparisons.

When tissue gastrin concentrations following different periods of omeprazole treatment were compared with concentrations in controls, Student's t-test was used for comparison between groups.

In paper III, comparisons between treated animals and controls were performed using the non-parametric Mann-Whitney U-test. When daytime and nighttime secretions within the same animal were compared and differences in *in vitro* experiments were analyzed, the non-parametric Wilcoxon signed-rank test was used.

In paper IV, comparisons between gonadectomized or sex hormone treated animals and controls were performed applying the non-parametric Mann-Whitney U-test. In all studies, differences were considered as statistically significant when $p \leq 0.05$.

RESULTS

Acid secretion studies

Inhibition of basal and stimulated acid secretion by omeprazole and ranitidine

In experiments using different doses of omeprazole, ranitidine or the vehicle Methocel, the effects on basal or stimulated acid secretions induced by pentagastrin or histamine were studied.

The effects of short-term administration of omeprazole (6 doses) and ranitidine 4 doses) are illustrated in table I. The average inhibition for omeprazole during 12 hours and for ranitidine during 6 hours are shown.

Table I

Inhibitory drug	Dose in μmol/kg	Average inhibition in %±SEM
Omeprazole	20	54±10
	30	63±10
	40	71±8
	80	94±4
Ranitidine	125	64±18
	187.5	76±15
	250	89±9
	375	92±5

In another series of experiments the effects by two doses of omeprazole (20 and 80 μmol/kg x 2) or ranitidine (125 and 375 μmol/kg x 4) were studied for 12 and 6 hours (table II) .

Table II

Inhibitory drug	Dose in μmol/kg	Inhibition in % ± SEM		
		2-3 h	6.5-7.5 h	11-12 h
Omeprazole	20	90±5	68±7	7±10
	80	100±0	98±1	54±11
		2-3 h	5-6 h	
Ranitidine	125	85±6	20±7	
	375	93±2	44±12	

In still other experiments, the effects on pentagastrin stimulated (650 nmol/kg/h) acid secretion by a single high dose (400μmol/kg) and a lower dose (80μmol/kg) of omeprazole given twice per day were compared. In these experiments, the average 24 h inhibition was more pronounced with the lower doses, whether secretion was basal (NS) or stimulated ($p<0.01$). Inhibition with the two lower doses was inconsiderably less than 50 % on one occasion during the 24 hour period. Inhibition evoked by the single dose, on the other hand, gradually faded after the 16 h observation period, whether the acid secretion was basal or stimulated.

In further experiments, basal and histamine-stimulated (1.1 and 136 μmol/kg) acid secretion before, during and after treatment with omeprazole (80 μmol/kg x 2) or ranitidine (375 μmol/kg x 4) were investigated. In addition to study the effect of the two inhibitory drugs on histamine stimulated acid secretion, the experiments were carried out to see whether secretions to threshold and maximal stimulation with histamine were influenced after that the treatment was finished. Omeprazole abolished both basal and stimulated acid secretions during the treatment period. When secretion was stimulated by the threshold dose of histamine, a significant ($p<0.05$) increase of acid secretion was noted 1 and 4 weeks after that treatment had ceased. When secretion instead was inhibited by ranitidine, the inhibition of basal secretion and secretion stimulated with a threshold dose of histamine stimulation was profound, although the secretions were not abolished. Secretion evoked by the maximal dose of histamine was partially inhibited by ranitidine. No significant changes in the post-treatment pattern of acid secretion was found, although a tendency to an increased acid output following stimulation with the threshold dose of histamine was noted.

A study was also performed in order to compare the acid inhibition of basal and pentagastrin (650 nmol/kg) stimulated acid secretion during 24 hours after repeated administration of omeprazole (80 μmol/kg x 2) and ranitidine (375 μmol/kg x 4). Omeprazole inhibited the 24 hour basal and pentagastrin stimulated secretion with 80±5 % and 78±2 %. The corresponding inhibition for ranitidine was 82±2 % and 79±1 %. The profile of the 24 hour inhibition is illustrated in fig 1.

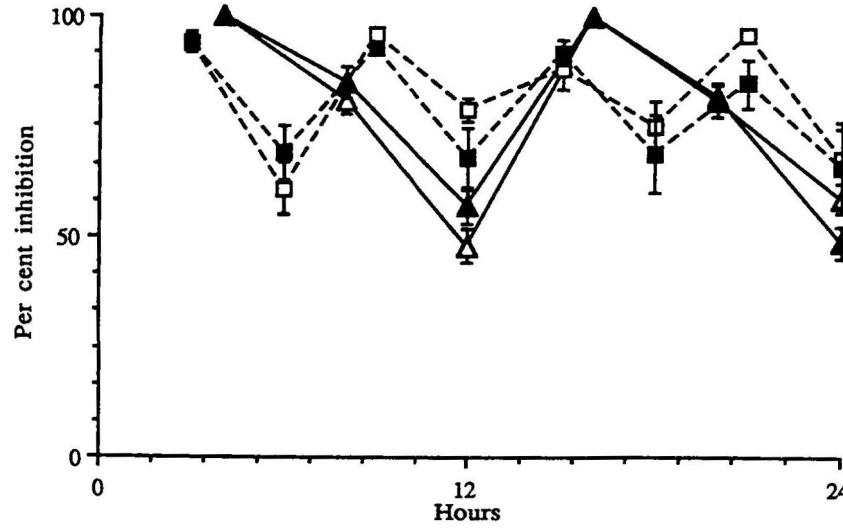

Fig 1. Basal (Δ, □) and pentagastrin (650 nmol/kg) stimulated (▲, ■) acid secretion during the seventh day of treatment with omeprazole (——, 80 μmol/kg) given at hours 0 and 12 and with ranitidine (- - -, 375 μmol/kg) given at hours 0, 6, 12, and 18 (n=6).

Gastrin studies

Studies in order to establish optimal conditions for gastrin studies after treatment with acid inhibitory drugs

In one type of experiments, rats were treated with omeprazole (80 μmol/kg) twice daily for 2 weeks and animals were killed 12 hours after the last dose. Before killing they had been subjected to fasting for 0-24 hours. Whereas average gastrin concentrations were higher than 800 pg/ml at the time (0) up to which the animals had been freely fed, gastrin concentrations then gradually decreased as fasting was prolonged and was approximately 125 pg/ml after 24 hours of fasting.

In another type of experiments, the rats had free access to food until they were killed, whereas the period between the last dosing with omeprazole and blood sampling varied between 0-24 hours. A certain increase in the plasma gastrin concentration could be seen during the first sampling periods (2 and 6 h). After that gastrin concentrations gradually decreased.

However, the decrease was considerably less than the decrease noted in the fasting experiments.

In still other experiments carried out in the animals mentioned above, gastrin concentrations in the gastric tissue were determined. Although certain variations in gastrin concentrations were noted on the different sampling occasions, no significant differences were found in experiments in which the periods of fasting 12 hours after last dosing were varied. When animals instead had free access to food and the period between last dosing and sampling was prolonged, a moderate but not statistically significant increase in tissue concentrations was noted after some time.

Tissue gastrin concentrations following 28 days of treatment with two doses of omeprazole (20 and 80 μmol/kg/day) and ranitidine (125 and 375 μmol/kg/day) and following another 28 days of recovery

The higher doses of omeprazole and ranitidine significantly increased the gastrin concentration and the increase was more pronounced in animals treated with omeprazole. The lower dose of omeprazole also raised the gastrin concentration but this increase was not significant. The low dose of ranitidine did not influence the gastrin concentration at all. After the recovery period for 28 days, gastrin concentrations in ranitidine treated animals did not differ from controls. Concentrations in omeprazole treated animals were only slightly raised.

Studies of sex hormones, acid secretion and gastrin

24 hour basal and stimulated acid secretion in normal male and female rats

Basal acid output remained at a similar level throughout the 24 hour period and was of the same magnitude in both male and female rats. Pentagastrin (500 μg/kg/hour) stimulated acid secretion, on the other hand, was higher in male rats (34%, $p<0.01$), but the difference was less pronounced at night.

24 hour basal and stimulated acid secretion in male and female control rats and in rats following gonadectomy.

Ovariectomy did not cause any significant effects on basal or stimulated acid secretion.

In male rats, orchidectomy slightly (14%) lowered the basal acid output during daytime and somewhat more at night (22%, $p<0.05$). The stimulated acid output was more reduced, 37% ($p<0.01$) during the day and less (18%, NS) at night. The 24 hour reduction of acid secretion was 29% ($p<0.01$).

Basal and stimulated acid secretion in male and female control rats and in rats after long-term treatment with estradiol

When female rats were treated with estradiol, a certain reduction (22-24%, NS) of the basal acid secretion was found during all observation periods. Stimulated acid secretion was suppressed by estradiol during daytime (29%, $p<0.01$). At night, secretion became 17% (NS) higher than in controls and secretion was significantly higher ($p<0.05$) than the daytime secretion within the same experiment.

Basal secretion in male rats was essentially the same as in untreated controls. Stimulated secretion, on the other hand, was considerably reduced (42%, $p<0.01$) during daytime. As was found in female rats, secretion at night was higher than in controls (32%, $p<0.05$) and significantly ($p<0.05$) higher than the daytime secretion within the same experiment.

Basal and stimulated acid secretion in male and female control rats and in rats following long-term treatment with testosterone.

Testosterone treatment of female rats reduced the basal acid secretion during both daytime (49%, $p<0.01$) and at night (33%, $p<0.01$) and the increase during the night, that was seen within the same experiment, was statistically significant ($p<0.05$). Stimulated acid secretion was also reduced during daytime (33%, $p<0.01$) in the female rats. At night, secretion was significantly increased ($p<0.05$) in comparison with day-

time secretion within the same experiment and also somewhat raised (9%, NS) when compared to nighttime secretion in control rats.

In male rats, testosterone treatment did not significantly influence the basal acid output but stimulated acid secretion was suppressed during daytime (30%, NS). When secretion was stimulated, testosterone influenced the acid output as in female rats, i.e. the acid output was higher during the night, whether the secretory level was compared with the daytime secretion within the same experiment ($p<0.05$) or nighttime secretion in control rats 36%, $p<0.01$).

Effects of estradiol or testosterone on aminopyrine accumulation in isolated male and female parietal cells

Incubation with various concentrations (10^{-9}-10^{-4} M) of estradiol or testosterone reduced ($p<0.05$) the AP accumulation induced by histamine (10^{-4}M) in male and female isolated parietal cells.

Number of gastrin cells in normal and gonadectomized rats and in rats treated with estradiol or testosterone

In normal rats, females had a somewhat higher number of gastrin cells (28%, N.S.) than males. Gonadectomy significantly reduced the number of cells in female rats (36%, $p<0.01$), whereas the decrease in cell number in male rats was less pronounced (14%, N.S.).

Estradiol treatment also reduced the number of gastrin cells in both females (34%, $p<0.01$) and males (36%, $p<0.05$). A similar effect was noted also when female (26%, $p<0.05$) and male (21%, N.S.) rats were treated with testosterone.

24 hour plasma gastrin concentrations in normal and gonadectomized rats and in rats treated with estradiol or testosterone

Plasma gastrin concentrations were similar in normal freely fed female and male rats when determined every 4th hour during a 24 hour period.

When the female rats were subjected to ovariectomy, nighttime (31%, $p<0.01$) and 24 hour (21%, $p<0.01$) gastrin concentrations were lower

than in controls. Orchidectomy produced an even more pronounced daytime (44%, $p<0.001$) and 24 hour (37%, $p<0.001$) reduction of the gastrin concentration in plasma.

When animals were treated with estradiol, there was instead an increase in the gastrin concentration in both females (26%, $p<0.05$) and males (23%, $p<0.05$). At night, gastrin levels in females were essentially unchanged (4% increase, N.S.). In males, on the other hand, gastrin concentrations increased (24%, $p<0.05$) at night in comparison with the untreated controls.

Testosterone treatment significantly lowered the 24 hour gastrin concentration in females (26%, $p<0.05$). In males, a more moderate (10%, N.S.) reduction was found.

DISCUSSION

The wide use of drugs producing a profound and long-lasting inhibition of acid secretion, the long term use of these drugs in ulcer treatment and the demonstration of frequent gastric tumours in female but not in male rats following long-term administration of omeprazole, motivate the present studies. The investigation in first hand focus on effects exerted by the H^+K^+-ATPase inhibitor omeprazole and on effects on acid secretion and gastrin parameters evoked by the sex hormones estradiol and testosterone. In several respects the omeprazole effects are compared with those of the H_2-receptor antagonist ranitidine.

Initial studies (I) were performed in order to compare the inhibitory effects by different doses of omeprazole and ranitidine on basal secretion and secretion induced by maximal pentagastrin stimulation. From these results, doses of the two drugs, producing similar inhibition of the acid output, were selected for studies of other problems associated with drug inhibition.

For example, it has been claimed in some human (Sewing et al. 1978; Frislid, Aadland and Berstad 1986; Jones et al. 1988; Fullarton et al. 1989) and animal (Witzel et al. 1977; Sundell and Nilsson 1986; Larsson et al. 1988) studies, that treatment with acid inhibitory drugs may increase the capacity of the corpus mucosa to secret acid. It has also been suggested from studies in man (Nwokolo et al. 1990; Rogers et al. 1989) that the inhibitory effect of H_2-receptor antagonists may be fading following some time of treatment. Experiments in the present study (I) were therefore designed to investigate the possible occurrence of tachyphylaxis and post-treatment acid hypersecretion by omeprazole and ranitidine in rats. However, omeprazole and ranitidine produced a consistent inhibition throughout the whole examination period, and no elevation of the maximal histamine-stimulated acid output was noted for any of the two drugs, indicating that the two drugs in our experiments did not induce tachyphylaxis or raise the maximal ability to secret acid. When secretion instead was stimulated by a threshold dose of histamine, acid output was significantly increased 1 and 4 weeks after the treatment with omeprazole showing an increased sensitivity of the parietal cells for histamine stimulation. In other studies, an increased sensitivity to threshold doses of histamine has been seen in humans treated with

cimetidine (Aadland, Berstad and Granerus 1981). A raised acid secretion has also been found in rats (Larsson et al. 1988) and dogs (Sundell and Nilsson 1986) after treatment with omeprazole for 3 and 12 months.

Doses of omeprazole and ranitidine causing similar secretory inhibition (fig.1) were also used in order to compare the drug effects on concentrations of gastrin in plasma and in the stomach (I and II). Previous studies (Creutzfeldt et al. 1986; Larsson et al. 1986; Decktor et al. 1989; Ryberg et al. 1989) exist in which gastrin concentrations have been determined following treatment with acid inhibitory drugs. However, the results are difficult to interprete due to variations in sampling conditions. We therefore started our gastrin studies by evaluating how variations in sampling conditions influence gastrin concentrations in plasma and gastric tissue. In one series of experiments, the animals were allowed to fast for different periods (0-24 hours) before sampling and in another series the time (0-24 hours) between the last dose of the inhibitory drug and sampling was varied.

Prolonged fasting gradually and considerably reduced the plasma gastrin concentration. Our finding of reduced gastrin concentrations after prolonged fasting agrees well with results by others obtained from experiments in which rats have been starved (Lichtenberger, Lechago and Johnson 1975; Schwarting et al. 1986). Increased time intervals between the last dosing and sampling in freely fed rats also resulted in a decrease of the plasma gastrin concentration, which was less prominent than the decrease in fasting animals. However, concentrations of gastrin in the gastric tissue did not significantly change during the corresponding sampling periods. The results thus show that determination of plasma gastrin concentrations on single blood samples following treatment with acid inhibitory drugs may show manyfold increases or be essentially normal depending on the conditions under which the blood samples have been collected, whereas gastrin concentrations in gastric tissue remain relatively stable during the corresponding time periods. Determinations of tissue gastrin concentrations may therefore be preferred in investigations intended to study effects of chronic influences on gastrin parameters. If the plasma gastrin concentration is of interest to measure, it may be better to perform repeated gastrin determinations during 24 hours, as was done in the plasma gastrin studies in paper IV.

When the effects on tissue gastrin concentrations by omeprazole (20 μmol/kg x 2 or 80 μmol/kg x 2) and ranitidine (125 μmol/kg x 4 or 375 μmol/kg x 4) were determined following treatment for 28 days, the high doses of both drugs increased the gastrin content. A certain increase was also found by the low dose of omeprazole, but not with the low ranitidine dose. The increase with the high omeprazole dose was considerably higher than with ranitidine. The high doses of the two drugs have been shown to produce an equal inhibition of the acid secretion (fig.1), whether it is basal or maximally stimulated with pentagastrin (650 nmol/kg/hour). The results therefore indicate that omeprazole may influence the antral mucosa in other ways than by raising the intragastric pH. This concept is supported by results from acute experiments (Ohe et al. 1983; Ohe et al. 1986; Ohe et al. 1989) in which the intragastric pH has been kept neutral. Thus, omeprazole and cimetidine, but not famotidine, raised the plasma gastrin concentration. Results indicating gastrin release by acid inhibitory drugs also have been presented by Decktor et al. (1989).

In separate experiments, the time course of changes in tissue gastrin concentration was studied for 28 days following treatment with the high dose of omeprazole (375 μmol/kg). Already following 3 days, a significant increase of the gastrin concentration was found, which increased further after 7 days and then essentially remained at the reached level.

After conclusion of the 28 days experiments, using the two doses of omeprazole (20 and 80 μmol/kg x 2) and ranitidine (125 and 375 μmol/kg/ x 4), animals were allowed to recover for another 28 days. Following this period, animals treated with ranitidine showed the same gastrin tissue concentrations as in controls. In omeprazole treated animals, a certain increase in gastrin concentration was found, but this elevation was not statistically significant. If the treatment period with omeprazole is prolonged (10 weeks), a longer recovery period is required until gastrin values are normalized (Larsson et al. 1986).

As mentioned previously, carcinoid tumours have been found in female rats following treatment with omeprazole using very low doses of omeprazole and with an increasing frequency when doses are raised. In male rats the frequency was much lower and tumours were only found at the highest omeprazole concentrations (Information från Socialstyrelsens

Läkemedelsavdelning, 1988). These findings raise the question whether sex hormones may play a role in the development of tumours at omeprazole treatment. As a beginning of such studies, we investigated how the sex hormones estradiol and testosterone influence some of the exocrine and endocrine secretions of the stomach. Some older studies have been performed in order to examine how acid secretion is influenced. As emphasized in the introduction, the results from those studies are sometimes conflicting. Several of them have been obtained from experiments carried out during a few hours and with the animals anaesthetized and, mostly, acid secretion has not been determined quantitatively but qualitatively by recording the pH of the gastric perfusates. (For references see pages 9-10).

The present studies were therefore carried out on animals provided with a chronic gastric fistula and basal as well as stimulated acid secretions were studied during 24 hours. Gonadectomies were performed very soon after birth and treatments with the sex hormones estradiol and testosterone were carried out for 3 months.

Our results show that the 24 hour basal acid secretion is of the same magnitude in normal male and female rats. Despite other experimental designs and much shorter periods of observations (2-6 h) in the studies by Olowo-Okurun (1975), Adeniyi and Olowo-Okurun (1989) and Adeniyi (1991), there is a good agreement between their results on basal acid secretion and ours. Although basal secretion was similar in both sexes in our study, stimulated secretion was significantly higher in males, indicating a greater secretory capacity in this sex. A comparison of the basal and stimulated secretions between the two sexes therefore permit the conclusion that basal secretion in males makes use of a smaller part of the maximal secretory capacity than in females.

Interestingly, a difference was also found between stimulated acid secretions during daytime and at night, inasmuch as the daytime secretion was higher in both males and females. This difference cannot presently be explained. It was most likely not due to losses of fluid and electrolytes during the experiments, since losses were compensated for by continous subcutaneous infusions of Ringer's glucose.

Elimination of the sex glands in female rats did not significantly influence the basal acid output. This observation is essentially in accordance with the previous results reported by Olowo-Okurun (1975) and Adeniyi (1991). Nor was stimulated acid secretion significantly changed in our study after the ovariectomy.

In orchidectomized animals daytime basal secretion was slightly and nighttime secretion somewhat more reduced. When these results are compared with those of Olowo-Okurun (1975) and Adeniyi (1991) the results are similar, although the observed reduction was greater in our study. When secretion in our study was stimulated with pentagastrin, orchidectomized rats secreted a significantly lower amount of acid during daytime. These results can be compared with those of Maitrya and Maitrya (1979), who observed reduction of histamine-stimulated acid secretion in orchidectomized and pylorus ligated rats studied for 2 hours. At night, the secretion in our experiments was only slightly lowered in comparison with the controls. The 24 hour secretory patterns in our orchidectomized animals were thus different whether the secretion was basal or stimulated.

When animals were treated with the sex hormones estradiol or testosterone in this study, basal secretion was lowered in females but not influenced in males. The stimulated acid secretion was reduced in both sexes during daytime but was higher at night, whether the secretion was compared with the daytime acid output within the same experiments or with the nighttime secretion in the control animals. Administration of the sex hormones thus influenced basal secretion differently in the two sexes, but stimulated secretion in a similar way and the effects on stimulated secretion at daytime was opposite to the effect observed at night.

The present study cannot offer any final explanations to the observed changes. Some of the differences may be ascribed to changes in the parietal cell mass. Adeniyi (1991) found that the parietal cell mass was 29 % lower in normal female rats. This finding did, on the other hand, not correspond too well with his observations on acid secretion. However, he was measuring basal acid secretion, that, according to our results, is of similar size in both sexes but makes use of different portions of the maximal secretory capacity. In our opinion, differences in the parietal cell mass should be compared to the maximally stimulated acid output,

which in our study was 34% higher in the males. Following orchidectomy, Adeniyi (1991) noted a 27% reduction in the parietal cell mass in the castrated animals, which corresponds to a 37% decrease in the maximal acid output in our study. However, the results obtained after ovariectomy in our study are difficult to relate to changes in the parietal cell mass. Adeniyi (1991) found that the parietal cell mass after ovariectomy was 32% higher than in normal female rats, whereas ovariectomy in our study did not cause any effects on acid secretion, whether secretion was basal or stimulated. Our results after administration of sex hormones cannot be compared with parietal cell mass studies, since such investigations do not seem to have been performed.

It should, however, be emphasized that it may be to simplify the explanations of our results too much by referring them exclusively to losses or additional effects by sex hormones. Thus, removal of the sex glands may eliminate factors other than the sex hormones that may influence the parietal cells. Also, removal as well as additional administration of sex hormones may influence the secretion of hypophyseal LH as well as other physiological factors that may exert an effect on the acid secretion. Another complicating factor in interpreting the results is that testosterone becomes metabolized to estradiol when given to male rats.

In order to avoid some of the complicating factors related above, the effects by the two sex hormones were studied on histamine-stimulated AP-accumulation in male and female rat parietal cells. As found in the in vivo experiments during daytime, both hormones inhibited the stimulation of the parietal cells.

In other in vivo studies in rats, the same experimental design as in the acid secretion experiments was used in order to investigate the effects by the sex hormones on the plasma gastrin concentration and the number of gastrin cells. Freely fed normal male and female rats had similar plasma gastrin levels (132±4 and 126±3 pg/ml). Unfortunately, these results are difficult to compare with results from other studies. Different sorts of substances have been administered to the control animals in those studies, gastrin concentrations have been determined on plasma samples collected on single occassions, whereas in the present study repeated determinations were performed during 24 hours from the same animals. In addition,

there seem to be considerable variations in gastrin concentrations measured by the different laboratories.

After gonadectomy, there was a decrease in both the number of gastrin cells and the plasma gastrin concentration in male and female rats. Our studies on acid secretion show that gonadectomy does not influence the acid output in females and moderately reduces the acid output in males. The lowered plasma gastrin concentrations were therfore most likely due to the reduction of the number of gastrin cells.

In other studies, it has been shown that gastrin cells are rare at birth and then gradually increase in number until the rats are 25-30 days old (Ekelund et al. 1985). In our study, antral tissue was collected much later and the results therefore give reason to the assumption that the gonads secrete some factor that is essential for the development of the normal gastrin cell density. When the animals were treated with estradiol the effects on the number of gastrin cells and plasma gastrin concentrations were divergent. The number of gastrin cells was reduced and the plasma gastrin concentration was increased at night in females and both during daytime and nighttime in males. Since gastrin secretion is dependent of the intra-antral pH, the observations have to be related to our previous acid secretion studies. In those studies, estradiol decreased the acid output during daytime and secretion increased at night and was then even larger than in controls. A lowered acid secretion is supposed to favour gastrin release and an increased acidity to reduce the secretion of gastrin. Considering these well established observations, it seems likely that other mechanisms than changes in the antral pH were operating after treatment with estradiol. The results may be given the interpretation that estradiol on one hand may reduce the number of gastrin cells and on the other act stimulating on the release of gastrin.

After treatment with testosterone, different effects were noted. As in the estradiol experiments, the number of gastrin cells was reduced but the plasma gastrin concentration was lowered in both sexes and the reduction was most pronounced in females.

In summary, surgical removal of the gonads reduces the number of gastrin cells and decreases the plasma gastrin concentration in both sexes. A decreased number of gastrin cells is also found when the rats are

treated with estradiol or testosterone. The plasma gastrin concentration is influenced differently by the two hormones, inasmuch as testosterone is decreasing and estradiol increasing the secretion.

CONCLUSIONS

1. Omeprazole and ranitidine effectively and dose dependently inhibit the basal acid secretion in the rat stomach. Oral administration of omeprazole twice per day and ranitidine four times per day may produce a similar 24 hour inhibition with the two drugs. The total omeprazole dose can be considerably reduced without loss of inhibitory effect, if the drug is administered twice instead of once daily. Maximal acid secretion stimulated with pentagastrin or histamine is more effectively inhibited by omeprazole than by ranitidine. No tachyphylaxis and no increase of the maximal acid secretion are developed after long term treatment with omeprazole and ranitidine, but the sensitivity of the parietal cells to stimulation with histamine is increased after four weeks of omeprazole treatment (I and II).

2. Fasting for 2-24 hours gradually lowers the plasma gastrin concentration raised by a high dose of omeprazole. Prolonged time between sampling of blood and the last drug administration decreases the plasma gastrin concentration in freely fed animals. The tissue gastrin content varies much less under the mentioned experimental conditions. Measurements of gastrin in antral tissue and/or repeated 24 hour determinations of the plasma gastrin concentrations are recommended, when gastrin changes are studied following long term influences on the gastrin mechanism (II).

3. Treatment with omeprazole and ranitidine for four weeks with doses that produce a similar degree of acid inhibition increases the gastric gastrin content. The tissue gastrin content increases considerably more in the omeprazole treated rats, in which also the plasma gastrin concentration becomes elevated, suggesting that omeprazole both inhibits acid secretion and stimulates the release of gastrin from the gastrin cells. Omeprazole raises the gastric gastrin content already after 3 days and the concentration then gradually increases. After four weeks of recovery gastric tissue concentrations have returned to the level of the controls (I and II).

4. Normal male rats have a higher capacity than females to secrete acid. Stimulated daytime acid secretion is higher than the nighttime secretion in both sexes. Gonadectomy does not influence basal or stimulated acid

secretion in females but reduces the acid output in males and the stimulated acid output is more reduced during daytime than at night. Treatment with estradiol insignificantly influence the basal acid secretion in both sexes. Testosterone lowers the basal acid output in females but not in males. In both sexes, estradiol and testosterone lower the stimulated acid secretion during daytime but raise the secretion at night. Estradiol and testosterone also act inhibitory on the parietal cells, when investigated in in vitro experiments (III).

5. Surgical removal of the sex glands lowers the number of gastrin cells and the plasma gastrin concentration in both sexes. Treatment with estradiol and testosterone decreases the number of gastrin cells. The plasma gastrin concentration is reduced by testosterone and increased by estradiol (IV).

ACKNOWLEDGEMENTS

This work was carried out at the former Department of Physiology, Department of Animal Physiology and Centre for Reproductive Biology, Swedish University of Agricultural Sciences, Department of Medicine and Surgery, Huddinge University Hospital, Huddinge and the Department of Pathology, University Hospital, Uppsala.

I wish to express my deep and sincere gratitude to the members of the Department of Animal Physiology and in particular to the following persons:

Professor Göran Nilsson, my advisor. I cannot find words to thank him for introducing me to and giving me constructive guidance through this field of research, for his intellectual, moral and material support throughout the course of my stay at the department. His unfailing encouragment and enthusiasm has helped me to raise my inner strength to complete this thesis.

I have made extraordinary demands on my co-workers and friends, Izabella Janczewska, Bengt Romell, Andreas Sandin, Rein Seensalu, and Erik Wilander. I thank all of them for their support, valuable comments and friendship.

I wish to express my sincere thanks to Anita Berg, Carin Hult, Sören Johansson, Andrzej Madej, Margareta Topel, Annika Wagman, Christer Wallsten and Eva Werner for various kinds of support.

My thanks also go to Lena Norlén, Inger Lind and Ann-Catrin Johansson for taking good care of the animals.

My very special regards go to my mother Weizero Abrehet Gebrehiwot, my father Ato Girmay Abera and my brother Gebregergis Girmay. Their support and interest in my education have inspired me with strength to pass the obstacles and complete this thesis. I also wish to thank my sisters Gabriela, Yeman and Letay, my other brothers Gebremedhin, Aregawi and Gebreegziabher, as well as relatives and friends for thier support and encouragements.

Finally, this thesis would not have come to an end without endless patience and a never failing support from my wife Genet Teklebrhan, who together with my son Hyab Kinfe fill me with warmth and love.

This study was supported by grants from the Swedish Medical Research Council (04X-3521), the Swedish Society of Medicine, Ruth and Richard Julins foundation, Professor Nanna Svartz foundation,''Förenade Liv'' Mutual Group Life Insurance Company, Stockholm, Sweden. Omeprazole was generously supplied by the Hässle AB, Sweden.

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ISSN 1401-6257
ISBN 91-576-5412-3