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**GASTRODUODENAL MOTILITY DURING THE DEVELOPMENT
OF EXPERIMENTAL DUODENAL ULCERATION. THE EFFECTS
OF ENTERIC TRANSMITTERS AND ANTI-ULCER DRUGS.**

by

Allison E. McKay

**A thesis submitted to the School of Graduate Studies of the University of
Ottawa in partial fulfillment of the requirements for the degree of Doctor
of Philosophy.**


Department of Physiology

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Ottawa, Canada.

June 1993.

 **Allison E. McKay, Ottawa, Canada, 1993**



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ABSTRACT

Duodenal ulcer disease is a heterogenous disorder involving hypersecretion of gastric acid, impaired mucosal defense mechanisms and altered gastroduodenal motility. Currently, the most reliable and widely used model for investigating experimental duodenal ulcers is the cysteamine-HCl treated Sprague-Dawley rat. Cysteamine-HCl rapidly and consistently induces ulceration to the lateral aspects of the proximal duodenal wall. Moreover, these chemically-induced ulcers have remarkably similar pathological and morphological features to the human disease condition.

Although acid secretion and duodenal mucosal neutralization mechanisms are clearly important to the pathogenesis of experimental duodenal ulceration, subcutaneous administration of cysteamine-HCl in rats alters/disrupts normal gastrointestinal motility. In fact, these disturbances in gastrointestinal motility precede secretory changes and actual development of the ulcers.

Cysteamine-HCl induced duodenal ulcers are also associated with biochemical changes in certain neurotransmitter levels, including dopamine, 5-hydroxytryptamine, histamine and γ -aminobutyric acid (GABA) in central and peripheral tissues. The peripheral GABAergic system has been determined to be a very extensive system in the mammalian gastrointestinal tract, with GABA functioning as both a neurotransmitter and neuromodulator in the control of gastrointestinal function. Thus, intrinsic neural pathways involved in

gastroduodenal propulsive motor activity may be important in the pathogenesis of duodenal ulceration.

The results obtained from the present study demonstrated that modulation of the GABAergic system affects the development of experimental duodenal ulcer. Stimulation of peripheral GABA_A-receptors resulted in aggravation of cysteamine-HCl induced duodenal ulcer which was reversed by the specific GABA_A-receptor antagonist bicuculline and by the GABA_A-receptor coupled Cl⁻ channel ionophore blocker picrotoxinin. However, stimulation of GABA_B-receptor sites by the GABA_B-receptor ligand baclofen ameliorated the induced ulceration. Based on the known effects of these compounds on acid secretion and motor activity, it would suggest that the actions of GABA, GABA antagonists and baclofen on experimental ulceration may be related to enteric GABA_A- and GABA_B-receptor stimulation of gastroduodenal motility and/or gastric acid secretion.

The standard therapy for the treatment of clinical duodenal ulcers is the use of histamine H₂-receptor blockers, such as cimetidine. However, the relapse rate and recurrence of duodenal ulcers can reach as high as 80%. Clearly, a more appropriate therapy, addressing more than just gastric acid secretion, is needed in the treatment of this disease. In the present work, cimetidine was a poor anti-duodenal ulcer agent in comparison to the powerful, protective actions of the GABA antagonists and baclofen. The better protection offered by the GABA antagonists and baclofen on experimental ulceration may be related to the potential effects of these compounds on both gastroduodenal motility and acid

secretion, two factors important in the pathogenesis of duodenal ulcer disease.

Many laboratories are investigating new compounds for the treatment of duodenal ulcer disease. In this study, U74500A, a compound which belongs to a novel series of 21-aminosteroids collectively termed the 'lazaroids', was found to have anti-duodenal ulcer actions comparable to the GABA antagonists bicuculline and picrotoxinin, and the GABA_B-receptor ligand baclofen. Although it presently has not been established if U74500A exerts effects on gastric acid secretion, the present in vitro studies have shown this compound to interact/interfere with cholinergic muscarinic and serotonergic mechanisms.

These studies, taken together, suggest an important role for motility, in addition to acid secretion and reduced mucosal defense mechanisms, in the development of duodenal ulceration. An in vivo recording technique, using miniaturised extraluminal foil strain gauges, and a semi-automated computerized system for the analysis of gastroduodenal motor activity from conscious, unrestrained rats were developed. This recording method has allowed for the first time, both contractions and relaxations of smooth muscle to be recorded.

In agreement with previous in vivo studies, the administration of cysteamine-HCl caused rapid alterations in gastroduodenal motility. In the antrum, cysteamine-HCl induced larger amplitude, longer duration contractions while exerting inhibitory actions on relaxations. These findings contrasted earlier in vivo studies which showed cysteamine-HCl to abolish gastric contractile activity. Similar to the present study, previous in vitro studies have demonstrated that cysteamine-HCl

evoked contractions of the rat stomach.

In the duodenum, cysteamine-HCl was found to have regional specific actions, inducing contractile hypermotility of the early proximal duodenum only. Although previous investigators have observed cysteamine-HCl to induce duodenal hypermotility, these studies did not observe any regional specific actions of this ulcerogen. In addition, in the present study, pretreatment with GABA resulted in modulation of the cysteamine-HCl induced effects on gastroduodenal motility.

Based on the current evidence, the early disruption of gastrointestinal motility in experimental ulceration appears to be important in the pathogenesis of duodenal ulcers. Correction of these motor disturbances may be beneficial in treating this disease. Moreover, the peripheral GABAergic system appears to function in the manipulation of duodenal ulceration. Drugs which target GABA_A- and/or GABA_B-receptor sites, and affect both acid secretion and motor function, may represent a potential new therapy for better treatment of duodenal ulcer disease.

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CHAPTER 1

Duodenal ulcer disease is a complex, multifactorial disease in which the etiology and pathogenesis is poorly understood. Duodenal ulcers are mucosal necrotic or regenerative defects which penetrate into the muscularis mucosa of the proximal duodenal wall (Szabo et al, 1982). It has been estimated that in the North American adult population, the lifetime prevalence of duodenal ulcer is approximately 8 to 10 percent while gastric ulcer is about 1 percent (Soll, 1989). Therapeutic approaches have focused on either anti-secretory or acid neutralizing compounds since these factors have been associated with the pathogenesis of duodenal ulcer disease. However, current therapies do not provide a cure but rather, simply reduce this disease below the symptomatic stage. In fact, recurrence of ulcers often occurs following cessation of anti-secretory drug therapy, indicating that gastric secretion is only one of several pathogenetic factors involved in this disease process. A more appropriate therapy which addresses all relevant etiological and pathogenetic factors is desperately needed for the treatment of duodenal ulcers.

1.1 Duodenal Ulcer Models.

The pathogenesis of this disease has been investigated using experimental

animal models. However, until the 1970s, only a few methods, including surgical techniques, neurological stimulation and chemical agents, produced duodenal ulcers in experimental animals, including the rat (Szabo, 1984).

Chemically induced duodenal ulcers include chronic pantothenic acid deficient diets in Sprague-Dawley (SD) rats (Seronde, 1963); infusion of secretagogues such as histamine, carbachol or pentagastrin in fasted SD rats (Robert et al, 1970; Eagleton and Watt, 1971); local application of acetic acid on the duodenum of SD rats (Okabe and Pfeiffer, 1971); the H₂ agonist dimaprit in the guinea-pig (Cho and Pfeiffer, 1981); reserpine and electrical shocks in the cat (Doteuchi, 1971); systemic administration of indomethacin plus histamine dihydrochloride in Donyru rats (Takeuchi et al, 1986), and the administration of diethyldithiocarbamate, a potent copper chelating agent, in SD rats (Niida et al, 1989; Takamasu et al, 1989; Teramura et al, 1989). Although each of these methods induced duodenal ulcers, the incidence of ulceration was often variable; in some cases, mortality was unacceptably high; and some methods required complicated procedures. Moreover, these chemically induced lesions lacked specificity, with ulcers often obtained throughout the gastrointestinal tract.

During an investigation of the toxic effects of alkyl compounds, Szabo and Selye (1972) and Selye and Szabo (1973) observed that both propionitrile and cysteamine-HCl (HS-CH₂-CH₂-NH₂) induced rapidly developing duodenal ulcers (24-48 hours) in Sprague-Dawley (SD) rats. Moreover, cysteamine-HCl induced duodenal ulcers more rapidly and with greater consistency than propionitrile (Selye

and Szabo, 1973; Szabo, 1978). Cysteamine-HCl induces solitary or double (opposing) duodenal ulcers upon oral or subcutaneous administration, without fasting or other preparation of the rat being necessary (Robert et al, 1974; Fujii and Ishii, 1975; Szabo, 1978). Using this model, ulcers develop 2 to 4mm from the pylorus on the anterior and/or posterior wall of the duodenum, with occasional perforation and/or penetration to the liver or pancreas (Szabo, 1978). The cysteamine-HCl rat model is an excellent, inexpensive and easily reproducible model for the study of duodenal ulcer disease, inducing ulcers with a similar pathology and morphology to that of the human disease condition, as illustrated in Table 1. Currently, the cysteamine-HCl treated SD rat is the most widely used animal model for chemically inducing duodenal ulcers for the study of pathogenetic factors and the testing of anti-duodenal ulcer agents (Selye and Szabo, 1973; Robert et al, 1974; Fujii and Ishii, 1975; Szabo et al, 1977; Szabo, 1978, 1984; Tanaka et al, 1985; Krantis and Nicholson, 1989; Tanaka et al, 1989, 1990; Pan et al, 1990; Krantis and McKay, 1991; Krantis et al, 1992).

1.2 Chemical Properties of Cysteamine-HCl.

Ulcer-inducing compounds have diverse chemical properties such that it has not been possible to correlate ulcerogenic potency with definite structure (Szabo et al, 1982). However, it has been recognized that compounds with two carbon backbones and one or two highly electronegative functional groups with

Table 1. Comparison of Duodenal Ulcer Induced by Cysteamine-HCl with Human Duodenal Ulcer. (+) = yes. (±) = variable. (Modified from Szabo, S. 1984. Laboratory Investigation. 51: 124).

<u>Characteristic</u>	<u>Cysteamine-HCl Duodenal Ulcer</u>	<u>Human Duodenal Ulcer</u>
(1) proximal duodenum	+	+
(2) perforate	+	+
(3) penetrate into liver and/or pancreas	+	+
(4) presence of chronic healed and/or active ulcers	+	+
(5) increased gastric acid output	+	+
(6) elevated basal serum gastrin levels	+	+
(7) supersensitivity of serum gastrin to food intake	+	+
(8) response to antacids	+	±
(9) response to antisecretory agents	+	±
(10) response to H ₂ -receptor antagonists	+	+

nucleophilic properties (-CN, -SH, -NH₂) are duodenal ulcerogens (Szabo et al, 1982; Giampaolo et al, 1991). Ulcerogenic activity is diminished by shortening, lengthening, branching, unsaturating, halogenating or hydroxylating the carbon chains (Szabo et al, 1982). In addition, Giampaolo et al (1991) have recently determined that the dipole moment, melting point and solubility in octanol are important characteristics for predicting duodenal ulcerogenic actions of chemical compounds.

Structure-activity studies have demonstrated a resemblance between chemical duodenal ulcerogens and certain putative neurotransmitters (including γ -aminobutyric acid (GABA), histamine, dopamine and acetylcholine), such that there is a common two or three carbon backbone which possesses, on at least one end, a potent nucleophile (N, O, C=O) (Szabo et al, 1982). These kinds of studies aid in the recognition of endogenous substances which may exert important modulatory influences on duodenal ulceration. In fact, extrapolations from structure-activity studies have led to the discovery and biochemical characterization of the anti-duodenal ulcer actions of dopamine, and suggested the involvement of GABA in duodenal ulcer disease (Szabo et al, 1982).

1.3 Histological Features of Cysteamine-HCl induced Duodenal Ulcers.

The development of cysteamine-HCl induced ulcers have been examined at the gross macroscopic and light microscopic levels (Poulsen and Szabo, 1977;

Rao and Joffe, 1979; Pfeiffer et al, 1987) and more recently, at the ultrastructural level (Pfeiffer et al, 1987; Pfeiffer et al, 1987). Ultrastructural studies have demonstrated that although the gross ulcers are localized to one or two predictable sites in the proximal duodenum, the precursors of cellular necrosis occur at multiple villous sites within a zone predestined for ulcer formation (Pfeiffer et al, 1987). Morphological intracellular changes occur as early as 30 minutes following the administration of cysteamine-HCl. The various stages of epithelial cellular degenerative changes have been described by Pfeiffer et al (1987) to "progress from an early stage of apical enlargement of endoplasmic reticula and loss of cytoplasmic-ground substance to early and minor mitochondrial cristae disruption and minor changes of intact microvilli, to later severe mitochondrial disruption, loss of microvilli and subsequent expulsion of cellular contents". These observations provided evidence that intracellular cytotoxic reactions occur early at the villous tips and may precede attack of the duodenal mucosa by intraluminal damaging factors.

Scanning electron and light microscopy studies (Poulsen and Szabo, 1977; Tanaka et al, 1986; Pfeiffer et al, 1987) observed cytologic lesions at the villous tips two hours following cysteamine-HCl treatment. Tanaka et al (1989) observed cysteamine-HCl induced epithelial cell damage of the duodenal mucosa within 30 minutes. Within eight to twelve hours, these lesions progressed to erosions with increased precipitation of mucus in the pre-ulcer area, desquamation of necrotic absorptive cells resulting in congestion and edema of the lamina propria, and exposure to luminal contents. Gross duodenal ulcers were evident at 20 to 24

hours due to the progression of the necrosis towards the crypts such that an avillous region occurred followed by the development of a crater. Therefore, cysteamine-HCl induces cellular damage simultaneously at multiple sites within the pre-ulcer zone rather than at a single site which subsequently enlarges (Poulsen and Szabo, 1977; Szabo, 1984; Pfeiffer et al, 1987; Pfeiffer et al, 1987). However, the initiating factors responsible for the subsequent development of duodenal ulcers remains unknown.

1.4 Healing of Cysteamine-HCl Induced Duodenal Ulceration.

The healing time as well as the mechanism of healing of cysteamine-HCl induced duodenal ulcers has been found to vary depending on the transmural depth of ulceration (Poulsen et al, 1985). The duodenal mucosa is composed of intestinal villi and crypts of Lieberkühn, with the proliferating cell zone of the crypts functioning to supply cells for the regeneration of the intestinal villi (Leblond and Messier, 1958; Quastler and Sherman, 1959). Poulsen et al (1985) observed re-epithelialization from the remaining parts of the crypts of Lieberkühn of the duodenal mucosa in cysteamine-HCl induced erosions within three days, and the mucosal surface was completely normal within 15 days.

In non-penetrating ulcers, contraction of the circular smooth muscle layer was determined to be important in the healing process, allowing the edges of the ulcer to approach one another, and to re-establish a complete layer of the

Brunner's glands which are important in the defense mechanisms of the duodenal mucosa, within 15 days (Poulsen et al, 1985). The healing time of penetrating ulcers was considerably prolonged (at least 50 days) compared to non-penetrating ulcers, with re-epithelialization and formation of Brunner's glands occurring from the ulcer margins (Poulsen et al, 1985; Fuse et al, 1988). Furthermore, Fuse et al (1988) provided additional evidence that the Brunner's glands, which are localized primarily to the duodenal submucosa and secrete mucus, HCO_3^- and epidermal growth factor, proliferate at the ulcer margins and together with the crypts of Lieberkühn contribute to the healing process in cysteamine-HCl induced ulceration.

More recently, Pan et al (1990) demonstrated that deep ulceration induced by cysteamine-HCl treatment was replaced by scar tissue and one layer of regenerated epithelium seven days following the injection. These studies by Pan et al (1990) found that it took approximately three times longer for an initial scarred ulcer to develop into a mature scar than an active ulcer to heal to the initial scarred stage. However, the regenerated mucosa took five times longer to reach a mature state than for the active ulcer to heal to the initial scarred state, with the central portion of the regenerated mucosa most sensitive to subsequent damage. This finding was an important discovery because in many duodenal ulcer patients, pharmacological therapy is stopped shortly after endoscopy shows healing of the ulcer but yet, many of these patients will experience a recurrence of the ulcer. Thus, in many situations, the complete healing process of the duodenal ulcer has

not occurred.

1.5 Secretory Changes in Cysteamine-HCl Induced Duodenal Ulcer.

Increased gastric acid secretion and decreased mucosal resistance are believed to be two major factors in the development of duodenal ulcer disease. However, many duodenal ulcer patients do not show hypersecretion of gastric acid, but are either normal or hyposecretors. Thus, enhanced acid secretion cannot be the sole factor in the development of duodenal ulcers.

1.5.1 Gastric Acid Secretion.

Cysteamine-HCl has been found to increase gastric acid secretion (Groves et al, 1974; Ishii et al, 1976; Szabo et al, 1977; Tamaki et al, 1978; Kirkegaard et al, 1980; Tanaka et al, 1986); stimulate pepsin secretion (Tamaki et al, 1978) and decrease or impair duodenal HCO_3^- secretion (Kirkegaard et al, 1981; Adler et al, 1983; Bridén et al, 1985; Ohe et al, 1988). These studies have shown that cysteamine-HCl induces a marked and prolonged (up to 11 hours) increase in gastric acid secretion, with maximal secretion occurring between two and five hours (Ishii et al, 1976; Szabo et al, 1977; Kirkegaard et al, 1980; Tanaka et al, 1986). In addition, Kirkegaard et al (1980) determined that the hypersecretion of gastric acid was dependent on intact, vagal innervation since following vagotomy,

the cysteamine-HCl induced effects on acid secretion were reduced. Moreover, factors in addition to acid hypersecretion are required for the development of duodenal ulcers since pentagastrin-induced secretion of gastric acid did not result in duodenal mucosal alterations.

In contrast to other studies, Robert et al (1974) described cysteamine-HCl induced inhibition of gastric acid secretion. However, differences in technique and a long, four hour pylorus ligation may explain the discrepancy in results between Robert et al (1974) and other investigators.

1.5.2 Alkaline Neutralization.

In the rat proximal duodenum, cysteamine-HCl impaired or decreased mucosal and pancreatobiliary alkaline secretions, and these alterations were present at 30 minutes but not after four hours (Ohe et al, 1982; Adler et al, 1983; Stiel et al, 1983; Bridén et al, 1985; Tanaka et al, 1986; Ohe et al, 1988). Bridén et al (1985) suggested that cysteamine-HCl may not affect the actual secretory process, but impair the ability of the mucosa to maintain high alkaline secretions in response to acid exposure. However, Stiel et al (1983) demonstrated that two key enzymes involved in duodenal mucosa HCO_3^- secretion, HCO_3^- -activated ATPase and carbonic anhydrase, were significantly decreased in cysteamine-HCl treated rats. Furthermore, alkaline phosphatase activity was also reduced, and this may reflect the activity of the HCO_3^- -activated ATPase in the brush border

membrane.

In 1987, Isenberg et al provided evidence that proximal duodenal mucosal bicarbonate output was markedly impaired in duodenal ulcer patients. Duodenal loop perfusion experiments in dogs (Harmon et al, 1978) and rats (Ohe et al, 1982; Isenberg et al, 1985) have shown that in the absence of pancreatic and biliary secretions, neutralization of gastric acid occurred through duodenal mucosal alkaline secretions, protecting the duodenal mucosa from injury.

Although the duodenal mucosa is important in protecting the duodenum against acid (Flemström and Garner, 1982; Flemström et al, 1982), the submucosal Brunner's glands also participate in the defense mechanisms of the duodenal mucosa (Kirkegaard et al, 1981; Fuse et al, 1988). Cysteamine-HCl has been shown to reduce the mucus-containing alkaline secretions from the Brunner's glands (Kirkegaard et al, 1981; Poulsen et al, 1981; Narasaki et al, 1985; Tsuchihashi et al, 1985). Studies have suggested that cysteamine-HCl may affect the synthesis rather than the release of secretions from the Brunner's glands since treatment with the ulcerogen caused flattening of the secretory cells; dilation of the acini and the absence of mucus content (Kirkegaard et al, 1981; Poulsen et al, 1981; Narasaki et al, 1985).

Although the volume of secretion from the Brunner's glands is relatively small, an impairment in the function of this gland may aid in the development of cysteamine-HCl induced duodenal ulceration. Moreover, the secretion of epidermal growth factor (EGF) from the Brunner's glands has been shown to have

cytoprotective actions on the rat duodenal mucosa (Kirkegaard et al, 1983). Following cysteamine-HCl treatment, these glands were depleted of EGF, suggesting that inhibition of EGF synthesis may represent another pathogenetic factor in experimentally induced ulcers. More recently, Fuse et al (1990) measured the thickness of Brunner's glands in patients with surgically resected duodenal ulcer, and observed hyperplastic changes of these glands which may represent a mucosal defense mechanism.

Secretory changes, including aggressive factors such as acid secretion and defense mechanisms, are clearly important in the pathogenesis of duodenal ulcer disease. However, these factors are not the precipitating conditions since these effects are not observed until at least 30 minutes following cysteamine-HCl treatment. In order to understand and treat this disorder, early pathogenetic factors must be determined and investigated.

1.6 Biochemical Changes in Cysteamine-HCl Induced Duodenal Ulcer.

1.6.1 Catecholamines.

Biochemical changes associated with duodenal ulceration can best be investigated in experimental animal models. Studies of structure-activity, pharmacological and biochemical relationships suggest that the catecholamines may play a role in experimentally induced duodenal ulcers (Horner and Szabo,

1981; Szabo et al, 1984; Gallagher et al, 1987; Oishi and Szabo, 1987; Szabo et al, 1987). In fact, structure-activity studies have shown certain similarities between cysteamine-HCl and the catecholamines (Szabo et al, 1982).

Administration of central/peripheral dopamine or peripheral noradrenaline decreased both the incidence and intensity of ulceration, suggesting that the actions of the catecholamines involved both central and peripheral receptor sites (Horner and Szabo, 1981). Cysteamine-HCl treatment induced time-dependent and dose-dependent changes in catecholamine levels in total brain, brain regions, stomach, duodenum, pancreas and adrenals in the rat (Szabo, 1987; Szabo et al, 1987). In both the rat brain and gastroduodenum, cysteamine-HCl caused an almost uniform depletion of noradrenaline, and augmented the severity of the induced ulceration (Horner and Szabo, 1981; Szabo et al, 1987). This effect of cysteamine-HCl on noradrenaline levels was maximal at four and seven hours following the administration of the ulcerogen and returned to normal within 24 hours (Szabo et al, 1987).

The changes in dopamine levels in response to cysteamine-HCl were found to be selective, with increased levels measured in the adrenals, a biphasic effect in the brain cortex, hippocampus and midbrain, and a uniform reduction in the glandular stomach and duodenum (Szabo et al, 1987). Dopamine and dopamine agonists, including bromocriptine, lergotril and apomorphine, have been shown to either prevent or reduce cysteamine-HCl induced ulceration whereas dopamine antagonists such as haloperidol, (-)-butaclamol and (-)-sulpiride aggravated the

experimental ulceration (Szabo, 1979; Gallagher et al, 1987).

Horner and Szabo (1981) demonstrated that α -methyl-p-tyrosine, an inhibitor of the rate-limiting enzyme tyrosine hydroxylase in catecholamine biosynthesis, also aggravated cysteamine-HCl induced duodenal ulceration. More recently, it has been found that pretreatment with the catecholamine precursor, L-tyrosine, which in normal rats increased dopamine but not noradrenaline levels in the brain cortex, corpus striatum, glandular stomach and duodenum, dose-dependently reduced the severity of the cysteamine-HCl induced ulcers (Oishi and Szabo, 1984; Oishi and Szabo, 1987). These results obtained by Oishi and Szabo (1987) and Horner and Szabo (1981) provided evidence that the effects of cysteamine-HCl may involve changes in gastrointestinal dopamine metabolism.

It has also been demonstrated in the gastric and duodenal muscularis propria that there are two times as many dopamine binding sites than in the mucosa, suggesting up-regulation of the dopamine receptors (Sandrock, 1981; Pihan et al, 1984; Glavin and Szabo, 1990). Moreover, Hernandez et al (1989) have recently provided preliminary evidence that dopamine receptor binding activity was increased in the duodenal mucosa but not antrum of duodenal ulcer patients.

Dopamine has also been observed to function as an inhibitory modulator of gastrointestinal motility, abolishing cysteamine-HCl induced hypermotility of the rat duodenum (Brouwers and Tytgat, 1980; Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Glavin and Szabo, 1990; Marzio et al, 1990). The protective actions of dopamine on experimentally induced ulcers may be a consequence of

its inhibitory actions on motility. Although both dopamine and noradrenaline have been found to be located in nerve terminals and varicosities in the myenteric plexus (Costa et al, 1976; Willems et al, 1985; Glavin and Szabo, 1990), evidence has been obtained which suggests that there are no dopamine, noradrenaline or adrenaline positive nerve cell bodies in the myenteric and submucous plexuses of the rodent small intestine (Ahlman and Enerbäck, 1974).

1.6.2 Serotonin.

Serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter stored in mucosal enteroendocrine cells and intrinsic enteric neurons (Gershon and Erde, 1981), has also been proposed to be involved in the development of cysteamine-HCl induced duodenal ulceration. In the glandular stomach, forestomach and in extracts of brain, Szabo and Horner (1978) observed an increase in 5-HT concentrations, four hours after the administration of cysteamine-HCl. However, more recently, studies by Szabo et al (1987) demonstrated that following cysteamine-HCl treatment, 5-HT levels were relatively stable in brain, stomach, duodenum, pancreas and adrenal rat tissues.

1.6.3 Histamine.

Histamine has been well-established to have an important, physiological role in the control of gastric acid secretion. The location of histamine has been demonstrated to be species-dependent, present in enterochromaffin-like cells in the rat (Soll et al, 1981) and mast cells in man and dog (Soll et al, 1979). Recently, histamine has been proposed to be involved in the pathogenesis of duodenal ulcer (Szabo et al, 1979; Boesby et al, 1983; Kim et al, 1985). In 1983, Boesby et al observed that the gastric mucosal content of histamine and the activity of histidine decarboxylase were increased following cysteamine-HCl treatment, and correlated with the severity of the induced ulcers. Kim et al (1985) demonstrated that inhibitors of histamine release (cromolyn sodium and tranilast) prevented cysteamine-HCl induced acid hypersecretion. These results together suggest that cysteamine-HCl induced changes in gastric secretion may be partly mediated by the release of histamine. Szabo et al (1979) demonstrated that the depletion of histamine or the administration of H₂-receptor antagonists (cimetidine, metiamide) did not abolish, but significantly reduced the cysteamine-HCl induced ulceration. Although it is not clear whether these effects are mediated through peripheral (gastric) and/or central (cerebral, mesocortical) H₂-receptors, histamine appears to be associated with the pathogenesis of duodenal ulcer disease, through the stimulation of H₂-receptors (Szabo et al, 1979).

Currently, the histamine H₂-receptor antagonist cimetidine is a standard

therapy for both the short term and long term treatment of clinical duodenal ulcers (Bianchi Porro et al, 1980; Korman et al, 1980; Ippoliti et al, 1983; Damman et al, 1987; Nwokolo et al, 1991). However, duodenal ulceration has a relapse rate as high as 80% after cessation of cimetidine treatment (Korman et al, 1980; Ippoliti et al, 1983; Lane and Lee, 1988; Pan and Liao, 1990; Pan et al, 1991).

Pan and Liao (1990) examined the histological maturity of regenerating duodenal mucosa and the recurrence of ulcers following treatment with an H₂-receptor antagonist (cimetidine or famotidine). In their studies, no recurrence of duodenal ulcer was observed when the regenerating mucosa resembled normal, healthy mucosa. Thus, the recurrence of ulcers appears to be correlated with the histological state of the regenerating duodenal mucosa (Pan and Liao, 1990; Pan et al, 1991).

Taylor et al (1978) showed that the metabolism and elimination of cimetidine was similar in man, dog and the rat. Furthermore, cimetidine was excreted unchanged into the urine, independent of the route of administration. The effects of cimetidine on experimentally induced duodenal ulcer are controversial, with this H₂-receptor blocker either significantly reducing (Okabe et al, 1977; Szabo et al, 1979; Poulsen et al, 1986; Tanaka et al, 1989) or having no effect (Stiel et al, 1983; Narasaki et al, 1985) on the ulceration. Cimetidine may also exert acid-independent effects in the mucosa of the small intestine. Mucosal injury induced by bile acid (chenodeoxycholic acid) and independent of gastric acid secretion was reduced by cimetidine (ip, iv or po) (Erickson, 1990). However, cimetidine has little

effect on gastric motility in the rat or dog (Okabe et al, 1977).

1.6.4 Prostaglandins.

The prostaglandins have been shown to inhibit acid secretion and maintain the mucosal integrity of the gastrointestinal tract, such that abnormalities in the synthesis or release of the prostaglandins may be another pathogenetic factor in duodenal ulcer disease (Hawkey and Rampton, 1985; Malagelada et al, 1986; Rachmilewitz et al, 1986; Wilson, 1986; Bukhave et al, 1990).

There is some evidence that there may be a defect in duodenal mucosal prostaglandin generation in duodenal ulcer disease (Ahlquist et al, 1983; Malagelada et al, 1986). PGE₂, the most abundant arachidonic acid metabolite produced in the gastrointestinal mucosa, has been found to be reduced in patients with active duodenal ulcer (Rachmilewitz et al, 1986). PGE₂ stimulates mucus and duodenal bicarbonate secretion. Bukhave et al (1990) observed that duodenal ulcer patients not only have impaired proximal duodenal mucosal bicarbonate production and secretion but in response to luminal acidification, release greater amounts of PGE₂ into the duodenal lumen than normal subjects. From these observations, Bukhave et al (1990) suggested that the enhanced PGE₂ response to lumen acidification may represent a compensatory phenomenon in response to the decreased mucosal bicarbonate production.

The rat duodenum has been shown to produce PGE₂ (Kauffmann et al,

1988). In cysteamine-HCl induced ulceration, duodenal PGE₂ generation was either reduced (Kauffman et al, 1988) or unchanged (Tanaka et al, 1986). Moreover, the exogenous administration of PGE₂ reduced cysteamine-HCl induced duodenal ulceration (Tanaka et al, 1986; Kauffman et al, 1988). Wallace et al (1990) recently demonstrated that interleukin-1_β, a biological protein capable of stimulating endogenous prostaglandin synthesis, also reduced cysteamine-HCl induced duodenal ulcers. These studies, taken together, suggest a physiological role of endogenous prostaglandins in duodenal ulcer disease. Although the evidence suggests that the prostaglandins may be an important, anti-ulcer therapy, the healing responses of the ulcers to the prostaglandins are similar to that observed with H₂-receptor antagonists and antacids (Wilson, 1986).

1.6.5 γ-Aminobutyric Acid.

γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter of the brain (Meldrum, 1982), has recently been established to function as a neurotransmitter in the mammalian enteric nervous system, present in interneurons throughout the mammalian gastrointestinal tract (Krantis et al, 1980; Erdő, 1985; Jessen et al, 1987; Krantis and Clark, 1991; Krantis and Clark, 1991). From structure-activity studies, it was suggested that certain neurotransmitters, including GABA, may be modulators in the pathogenesis of duodenal ulcer disease (Szabo et al, 1982). It was demonstrated by Szabo et al (1982) that centrally (icv)

administered GABA or GABA agonists aggravated cysteamine-HCl induced duodenal ulcer whereas GABA_A-receptor antagonism exerted protective actions. In these studies, GABA was also found to cause adrenal necrosis. Moreover, stimulation of central GABA_A-receptor sites has been demonstrated to increase gastric acid secretion (Levine et al, 1981; Goto et al, 1985; Blandizzi et al, 1988). Based on these studies, centrally administered GABA may contribute to the enhanced gastric secretions associated with cysteamine-HCl induced duodenal ulcer.

GABA, its enzymes of synthesis and metabolism (glutamic acid decarboxylase and GABA transaminase), and high affinity uptake sites have been demonstrated to be present in the myenteric plexus of the guinea-pig, rat, cat and human (Furness and Costa, 1982; Erdö and Kiss, 1986; Krantis and Harding, 1986; Taniyama et al, 1987). GABA has been established as a modulator of intestinal motility, where its actions are mediated through bicuculline-sensitive, Cl⁻-dependent GABA_A-receptors on enteric cholinergic excitatory motor neurons and inhibitory non-adrenergic non-cholinergic (NANC) motor neurons and through bicuculline-insensitive GABA_B-receptors which prejunctionally inhibit the excitatory cholinergic motor neurons (Krantis et al, 1980; Krantis and Kerr, 1981; Giotti et al, 1983; Maggi et al, 1984; Krantis and Harding, 1986). Studies have shown peripheral GABAergic nerve-mediated stimulation of acid secretion (Levine et al, 1981; Harty and Franklin, 1983; Tanaka, 1985; Harty and Franklin, 1986; Blandizzi et al, 1988; Del Tacca et al, 1989). The presence and proposed roles of this

peripheral GABAergic nervous system suggested that this system may also be associated in the pathogenesis of duodenal ulcer disease. Studies by Krantis and Nicholson (1989) and Krantis and McKay (1991) provide convincing evidence for the involvement of peripheral GABA. In these studies, systemically administered GABA augmented cysteamine-HCl induced duodenal ulcer which could be dose-dependently reversed by the GABA antagonists, bicuculline and picrotoxinin.

More recently, GABA has been proposed to have neuroendocrine actions in addition to motor effects in the mammalian intestine, since GABA_A-receptors have been located on gland cells of the rat gastric mucosa and upper small intestine (Jessen et al, 1988; Erdö et al, 1989; Erdö et al, 1990; Gilon et al, 1990; Harty et al, 1991), and on intestinal enterochromaffin cells (Schwörer et al, 1989). However, the extent of involvement of peripheral GABA mechanisms to the development of duodenal ulceration remains unknown.

A number of biochemical alterations, including dopamine, GABA and histamine, have been associated with cysteamine-HCl induced duodenal ulcer. Although these alterations are important to the pathogenesis of duodenal ulcer disease, these changes cannot be the precipitating factors due to the delay in onset of effect following cysteamine-HCl treatment.

1.7 Effects of Hormones on Cysteamine-HCl Induced Duodenal Ulcers.

1.7.1 Gastrin and Secretin.

Both gastrin and secretin are affected in experimental duodenal ulceration. Cysteamine-HCl enhanced basal and food-stimulated serum gastrin levels and reduced plasma secretin (Lichtenberger et al, 1977; Kirkegaard et al, 1982; Szabo, 1984; McIntosh et al, 1984). Lichtenberger et al (1977) demonstrated a three to four fold increase in serum gastrin levels following cysteamine-HCl treatment and suggested that gastrin release may be responsible for the increased secretion of gastric acid which preceded the actual lesion development on the duodenal mucosal surface. Moreover, an intact vagus appears to be necessary for the cysteamine-HCl induced increase in serum gastrin. Increased gastric acid secretion in response to cysteamine-HCl has also been shown to be dependent on an intact vagus (Ishii et al, 1976). In addition, propranolol, a β -adrenergic antagonist, inhibited the cysteamine-HCl induced increase in serum gastrin whereas the anti-cholinergic blocker atropine had no effect, suggesting the involvement of β -adrenergic receptors (Kirkegaard et al, 1982). Adrenergic nerves have previously been shown to be present in the abdominal vagus of both the cat and man (Nielsen et al, 1969; Lundberg et al, 1976).

Increased gastrin secretion from isolated antral G cells has been shown to occur following the administration of cysteamine-HCl, suggesting that under in vivo

conditions, the effects of this ulcerogen on gastrin may be a direct action (Lichtenberger, 1982; Lichtenberger et al, 1982). Patients with clinical duodenal ulcer have been shown to exhibit increased sensitivity to gastrin (Lam and Koo, 1985).

In the rat, the cysteamine-HCl induced decrease in plasma secretin is prolonged, lasting from 30 minutes to three hours (Szabo, 1984). Secretin has been demonstrated to inhibit gastric acid secretion in response to pentagastrin, decreased serum gastrin and enhanced bicarbonate output. Failure to release secretin as a result of duodenal acidification could be an important factor in duodenal ulceration (Konturek et al, 1973; Itoh et al, 1975). In addition, secretin has been shown to inhibit antral motility (Misiewicz, 1976).

1.7.2 Somatostatin.

Somatostatin, a potent inhibitor of gastrin secretion, has been implicated in the pathogenesis of duodenal ulcer, abolishing cysteamine-HCl induced ulceration (Schwedes et al, 1977; McIntosh et al, 1984; Szabo and Reichlin, 1985). In the rat, somatostatin is located in both mucosa 'D' cells and intestinal neurons of the myenteric and submucosal plexuses (Furness and Costa, 1982). The administration of cysteamine-HCl has been found to deplete somatostatin in the gastrointestinal tract (stomach, duodenum and pancreas) and throughout the central nervous system (Szabo and Reichlin, 1981; Sagar et al, 1982; McIntosh

et al, 1984; Millard et al, 1985; Szabo and Reichlin, 1985) Szabo and Reichlin (1985) suggested that the somatostatin-induced depletion by cysteamine-HCl was caused by a conformational change in the molecular configuration, affecting the biological and immunological properties of this peptide. Using isolated vascularly perfused rat stomach, Short et al (1985) demonstrated that gastric somatostatin inhibited basal gastrin release and acid secretion by the gastric parietal cell and proposed that this regulatory peptide may have a local, regulatory role on the release of gastrin. In addition, the administration of exogenous γ -aminobutyric acid (GABA) was found to stimulate gastrin release and inhibit somatostatin release from isolated mucosal fragments of the rat antrum, affecting the release of both antral gastrin and somatostatin through stimulation of postganglionic cholinergic neurons in the antral mucosa and submucosa (Harty and Franklin, 1983, 1986).

1.8 Blood Flow Changes and Cysteamine-HCl Induced Duodenal Ulcer.

In studies examining the pathogenesis of duodenal ulcer disease, much attention has been focused on aggressive factors such as acid hypersecretion, with considerably fewer studies investigating mucosal defense mechanisms. Normal duodenal mucosal blood flow has been demonstrated to be important in protecting the mucosa against injury, with local ischemia proposed to be an early pathogenetic factor in duodenal ulcer disease (Szabo, 1984; Leung et al, 1985; Scremin et al, 1989). Local blood flow changes may be important in duodenal

ulcer development since blood flow has protective actions in the duodenum, removing hydrogen ions diffusing into the mucosa from the lumen and maintaining duodenal bicarbonate secretion (Dorricott et al, 1975; Scremin et al, 1989).

Current methods which are available for the measurement of duodenal blood flow include microspheres, hydrogen clearance and laser doppler velocimetry, but the results obtained from these studies are contradictory. Using various sized microspheres in the rat, Szabo (1984) observed a transient increase in total duodenal blood flow, which occurred 15 to 30 minutes following the administration of an ulcerogenic dose of cysteamine-HCl. However, measurement of duodenal mucosal blood flow with the hydrogen clearance method, showed reduced blood flow as early as 5 to 60 minutes following cysteamine-HCl treatment (Szabo, 1984; Kurebayashi et al, 1985; Kurebayashi et al, 1985; Tanaka et al, 1989). Tanaka et al (1989) also observed a decrease in duodenal blood flow using laser doppler techniques, and this reduction lasted approximately 60 minutes. There is also some evidence of diminished perfusion of the tips of the duodenal villi following the administration of cysteamine-HCl which coincides with increased vascular permeability of the duodenal mucosa (Szabo, 1984; Yabana et al, 1984).

Although recent studies present contradictory effects of this ulcerogen on duodenal blood flow, it is apparent that the induced changes in blood flow precede the development of lesion formation on the duodenal mucosal surface. Thus, impairment of the duodenal microcirculation appears to be yet, another factor

associated with duodenal ulcer disease. Mechanical activity of the smooth muscle of the gastrointestinal tract redistributes blood flow within the gut wall, and these effects together may affect the ulcerogenic process, development and location of duodenal ulcers (Fondacaro, 1984; Szabo, 1984).

1.9 Effects of Motility and Cysteamine-HCl Induced Duodenal Ulcer.

The proximal stomach consists of the gastric fundus and orad corpus and functions as a reservoir, allowing accommodation of food without large increases in intragastric pressure (Vantrappen et al, 1986). The distal stomach is composed of the distal corpus, antrum and antroduodenal regions, and is involved in the mixing, grinding and emptying of gastric contents (Vantrappen et al, 1986).

Motility of the stomach is due to activation of gastric smooth muscle cells which are arranged in three layers, an outer longitudinal layer, a middle circular muscle layer and an inner oblique layer (Weisbrodt, 1985). The stomach receives intrinsic innervation by neurons of the various enteric nerve plexuses within the gastric wall, with the myenteric plexus, located between the longitudinal and circular muscle layers (Figure 1), being the most prominent as well as extrinsic innervation from the sympathetic nervous system (Weisbrodt, 1985).

Motor activity of the stomach has been organized to allow propulsion of partially digested gastric contents into the proximal duodenum (Kelly, 1981; Weisbrodt, 1985). Slow, sustained or rapid, phasic contractions are the major

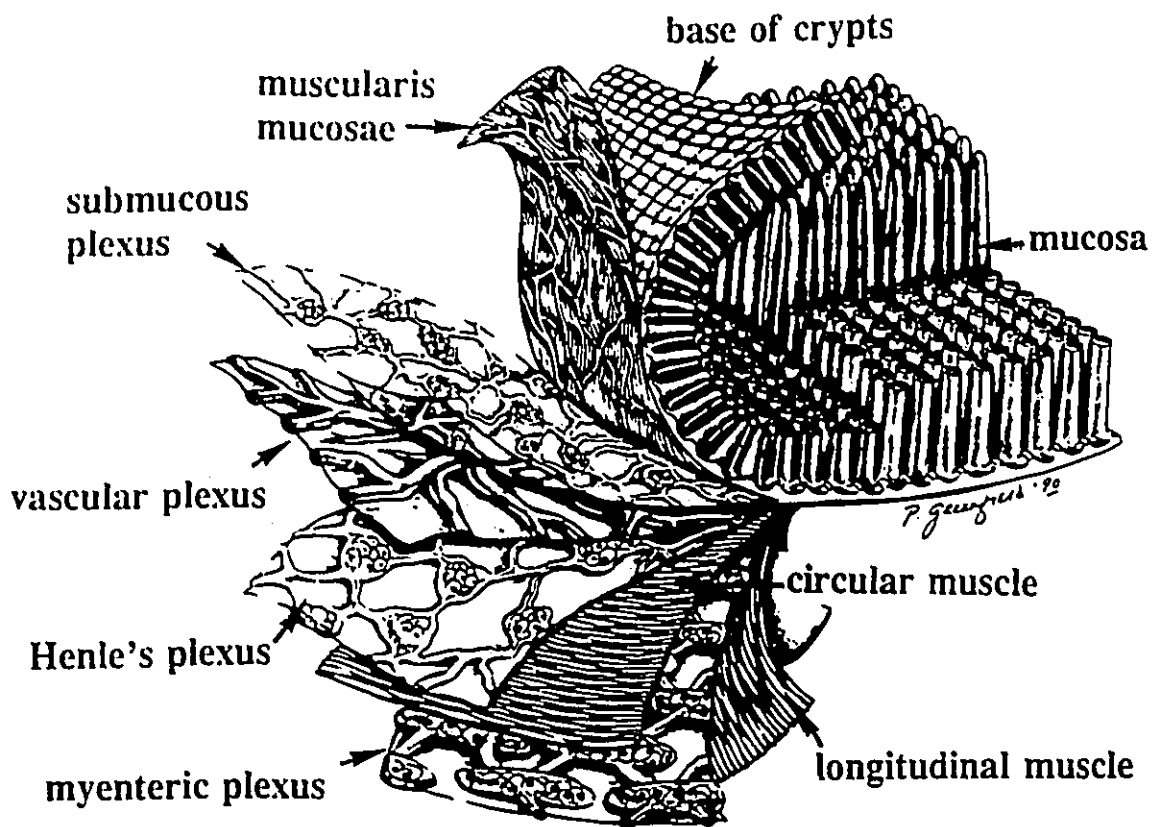


Figure 1. A schematic illustration of the wall of the gastrointestinal tract with the layers pulled back to show the general arrangement of the smooth muscle layers and nerve plexuses.

patterns of contractile activity present in the proximal stomach. However, in the distal portion of the stomach, the primary contractile event is the peristaltic contraction, which consists of a circular ring of contractions, increasing in both amplitude and velocity, but not frequency as it propagates distally towards the antrum. The end result of this type of motor activity is mixing and emptying of gastric contents (Kelly, 1981; Weisbrodt, 1985; Vantrappen et al, 1986).

Slow waves, also referred to as the basal electrical rhythm or pacemaker potential, are also present in smooth muscle cells of the distal stomach, where the membrane potential fluctuates rhythmically, resulting in cyclical depolarizations and repolarizations (Misiewicz, 1976; Kelly, 1981; Costa and Furness, 1982; Weisbrodt, 1985). These slow waves consist of an initial upstroke potential and secondary plateau potential, and are regarded as action potentials since they have the ability to initiate contractions (Weisbrodt, 1985). However, slow waves are always present, regardless of the presence or absence of contraction. Gastric emptying is modulated by alterations in gastroduodenal motility. In fact, abnormal gastric motility, due to a functional disorder of the stomach such as disorganization or disruption of normal motor events, has been associated with the rate of gastric emptying (Weisbrodt, 1985).

The small intestine, composed of the duodenum, jejunum and ileum, functions in the digestion and absorption of nutrients (Vantrappen et al, 1986). Motility of the small intestine has been organized to optimize digestion, such as mixing intestinal contents with digestive enzymes, and allowing propulsion of the

luminal contents to occur in an anal direction (Costa and Furness, 1982; Weisbrodt, 1985; Vantrappen et al, 1986).

Similar to the stomach, the small intestine receives both intrinsic and extrinsic innervation. Intrinsic innervation results from enteric neurons of the myenteric plexus and submucous plexus (located between the circular muscle layer and the mucosa, see Figure 1) (Weisbrodt, 1981; Costa and Furness, 1982; Weisbrodt, 1985). These two ganglionated and interconnected nerve plexuses are embedded within the intestinal wall, and function as a single, integrative system (Cooke, 1980; Lundgren et al, 1989). Extrinsic innervation of the small intestine is mediated through the vagus nerve and nerve fibres from the celiac and superior mesenteric ganglia.

Slow waves are also an important feature of intestinal smooth muscle cells. However, in contrast to the stomach, intestinal slow waves do not have the capability to initiate contractions but rather, represent cyclical changes in smooth muscle excitability (Costa and Furness, 1982). In vivo studies have demonstrated that slow waves are present throughout the small intestine, in both the circular and longitudinal muscle layers (Bass, 1968; Daniel, 1968; Costa and Furness, 1982). Slow waves are important in motility patterns of the intestine, with varying frequencies observed in different species (Ruckebusch and Fioramont, 1975; Stoddart et al, 1979). Furthermore, the frequency of slow waves decreases from the duodenum to the ileocecal region, with lengths of intestine showing similar frequencies (Costa and Furness, 1982). Hence the majority of slow waves tend

to propagate in an anal fashion.

Action potentials, also referred to as spike potentials, spike bursts or electrical control activity, are superimposed on slow waves, and are responsible for initiating contraction of intestinal smooth muscle (Bass, 1968; Daniel, 1968; Costa and Furness, 1982). However, action potentials do not propagate for more than 5mm along the intestinal muscle. Thus, series of action potentials are required for propagation of anally-directed contractions (Costa and Furness, 1982).

The neural control of gastrointestinal motility has been stated to be more complex than that observed with any other visceral muscular region (Davison, 1983). The small intestine is capable of eliciting series of highly coordinated contraction and relaxation responses to mechanical stimulation, resulting in the propulsion of gastrointestinal contents (Bayliss and Starling, 1899; Costa and Furness, 1982). Bayliss and Starling (1899, 1900, 1901), who initiated mechanical stimulation by insertion of a cotton-wool bolus into the intestine of rabbits, cats and dogs, termed this coordinated response the peristaltic reflex. Stimulation of the gut wall induces aboral propulsive movements which are independent of the central nervous system such that inhibition and relaxation precedes the bolus and excitation and contraction occurs behind the bolus (Bayliss and Starling, 1901). These distension-induced, excitatory and inhibitory motor patterns form the basis of the law of the intestine.

A second type of gastrointestinal motor activity observed by Bayliss and Starling (1901) were rhythmic, pendulum-like movements due to the simultaneous

contractions of both the circular and longitudinal smooth muscle layers. This pattern of motor activity is termed segmentation, and consists of two short standing, brief duration contractions which are separated by a short distance (Wingate, 1983). Moreover, segmentation involves reciprocal neural inhibition and disinhibition of adjacent muscular segments to prevent simultaneous contraction of the gastrointestinal tract (Wood, 1981).

During the interdigestive state, gastroduodenal motility is characterized by a cyclically recurring pattern of motor activity which is known as the interdigestive migrating motor complex (MMC) (Sarna et al, 1981, 1983; Schemann and Ehrlein, 1986; Vantrappen et al, 1986; Sarna, 1987). MMCs are present in the lower esophageal sphincter (LES), stomach and small intestine of most mammals, including the dog, rat and human (Sarna, 1985). Moreover, it has been proposed that MMCs are usually initiated in the LES and then distally propagated, but there are also other situations when the MMC is initiated in either the proximal or distal small intestine (Sarna et al, 1983).

In general, the MMC consists of four cyclical phases, which are similar in both the stomach and small intestine (Sarna et al, 1981; Sarna, 1985; Schemann and Ehrlein, 1986; Vantrappen et al, 1986). Phase 1 shows little if any contractile motor activity, and has been proposed to be the result of quiescent, enteric cholinergic neurons since the intestine is empty and hence, local reflex activity is absent (Weisbrodt, 1981). In phase 2 of the MMC, irregular contractions of the circular muscle, and an increased proportion of slow waves showing action

potentials has been observed. During the third phase, each slow wave has a superimposed action potential, resulting in regular spiking activity and regular contraction of the smooth muscle. Phase 4 is a very short phase, characterized by the disappearance of action potentials. This cyclical pattern of contractile activity is promptly abolished in the event of feeding, with no regular or predictable pattern of contractile activity (Sarna, 1987).

The physiological role of the MMC has been proposed to clear the small intestine of residual food, desquamated cells and other debris following the completion of digestion and to maintain bacteria overgrowth to the lower regions of the small intestine (Vantrappen et al, 1986; Sarna, 1987).

The pathogenesis of cysteamine-HCl induced duodenal ulcers clearly involves both aggressive and defensive mechanisms, but these neural, hormonal and secretory alterations/disruptions cannot explain the location or morphology of the experimental ulcers. Motor activity of the gastroduodenum is important for homeostasis of the upper gastrointestinal tract (Pihan et al, 1985). Moreover, the distribution of acid and pepsin in the duodenum as well as the contact with the mucosa is directly affected by gastrointestinal motility (Barbara et al, 1980). Mersereau and Hinchey (1984) have proposed a channel hypothesis to explain the location of experimental duodenal ulcers, suggesting that the smooth muscle of the pyloroduodenum may direct gastric effluent to two specific but opposing regions of the duodenum.

Kent et al (1975) observed in rats that injury to the small intestinal mucosa

resulted in an inhibitory effect on gastric emptying. In clinical studies of duodenal ulcer, alterations in gastric emptying have been observed, with many patients showing accelerated gastric emptying (Barbara et al, 1980; Malagelada and Larach, 1980; Harasawa and Miwa, 1982; Parr et al, 1988). A more rapid rate of gastric emptying would result in a larger than normal quantity of unbuffered acid being delivered into the duodenum. However, it is not known whether these alterations are caused by the ulcer itself or represent some underlying pathophysiological process (Harasawa and Miwa, 1982).

Cysteamine-HCl has been found to delay gastric emptying, resulting in a accumulation of gastric contents (Lichtenberger et al, 1977; Poulsen et al, 1982; Kline et al, 1988; Tanaka et al, 1989; Tanaka et al, 1990). Poulsen et al (1982) determined that the delay in gastric emptying was a result of pronounced relaxation of the stomach and abolishment of gastric peristalsis, which subsequently led to gastric retention and stimulation of acid secretion. Due to the absence of peristalsis, gastric contents were not mixed with the secreted acid, such that the gastric secretions were gradually emptied into the duodenum which has a diminished defense mechanism (Poulsen et al, 1982). However, studies by Kline et al (1988) demonstrated in rats that acute treatment with cysteamine-HCl delayed gastric emptying but prolonged exposure induced chronic ulcers with either unaltered or accelerated gastric emptying, similar to clinical studies. From these studies, it was suggested that the observed acceleration in gastric emptying may be an acquired alteration.

It has recently been suggested that motility and pressure alterations of the gastroduodenum, such as abnormal propulsion and mixing, may be of greater importance than alterations in gastric emptying, during the development of duodenal ulcers (Szabo, 1984; Szabo and Vattay, 1990). Gastroduodenal motility has been examined in patients with clinically diagnosed duodenal ulcer (Monto et al, 1974; Borgström and Arborelius, 1978; Sekiguchi et al, 1983; Bortolotti, 1989; Bortolotti et al, 1989; Kerrigan et al, 1991). Kerrigan et al (1991) recently demonstrated duodenal dysmotility in duodenal ulcer patients, observing increased duodenal retroperistalsis; diminished antroduodenal, aborally-directed pressure waves and increased incidence of atypical forms of duodenal motor activity. Evidence obtained from patients with duodenal ulcers shows inhibition of the migrating motor complex activity, which has been determined to be due to increased acid secretion, possibly prolonging the action of the acid on the duodenal mucosa (Bortolotti, 1989; Bortolotti et al, 1989). In addition, Monto et al (1974) found a decreased frequency of contractile waves in the proximal portion of the duodenum and increased frequency of waves in the distal duodenum of duodenal ulcer patients. However, there is relatively little information regarding motor function during the early stages of duodenal ulcer disease, when changes in the motility patterns could have significant etiological importance to the pathogenesis of duodenal ulceration.

The duodenal ulcerogen, cysteamine-HCl, has been observed to induce rapid and extensive alterations in gastroduodenal motility in the rat (Szabo et al,

1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). Moreover, these studies have demonstrated that the ulcerogen-induced alterations in motility patterns precede the secretory changes and ulcer development. Within five to 15 minutes of the administration of cysteamine-HCl (sc or iv), manometric pressure recordings of contractions of the stomach were reduced in the corpus and increased in the antropyloric region (Pihan et al, 1985). Large amplitude gastric contractions were almost completely abolished (Takeuchi et al, 1987). In addition, Pihan et al (1985) observed that cysteamine-HCl either had no effect, or caused a slight (up to 20%) increase in the frequency of antral polyphasic slow wave complexes.

In the rat duodenum, myoelectric recordings demonstrated that cysteamine-HCl caused an almost immediate (within 15 minutes) disruption to the myoelectric migrating complexes, with increased contractile spiking activity, indicating a state of hypermotility, which lasted for three to six hours (Szabo et al, 1984; Pihan et al, 1985; Mangla et al, 1989). These studies also observed a more slowly developing and prolonged decrease, up to 15 hours, in the frequency of slow waves. In addition, pressure recordings of duodenal motility provided further evidence of cysteamine-HCl induced disruptions, with enhanced contractions and hypermotility observed (Pihan et al, 1985; Takeuchi et al, 1987). The data obtained from these studies suggest that in experimental duodenal ulcer, there is an early phase of duodenal hypermotility followed by a longer lasting state of hypomotility (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989).

Studies have also demonstrated that dopamine has an inhibitory effect on cysteamine-HCl induced hypermotility which correlates well to the anti-duodenal ulcer actions of dopamine (Szabo, 1979; Szabo et al, 1984; Pihan et al, 1985; Gallagher et al, 1987; Takeuchi et al, 1987).

These myoelectric and motor alterations of the gastroduodenum, following the administration of the ulcerogen, are considered to be the earliest known abnormality in the development of duodenal ulcer disease, preceding secretory, biochemical and morphological changes.

The pharmacological actions of cysteamine-HCl have also been examined in the rat and guinea-pig small intestine under in vitro conditions, where it has both neurogenic and myogenic modes of action (Bakich et al, 1984; Pihan et al, 1985; Krantis, 1987; Krantis and Krause, 1989). In contrast to in vivo studies, cysteamine-HCl induced concentration-dependent contractions in isolated circular and longitudinal smooth muscle preparations of the rat stomach (Pihan et al, 1985; Krantis, 1987; Krantis and Krause, 1989). These studies have found that the gastric actions of cysteamine-HCl were predominantly myogenic and sensitive to cholinergic muscarinic antagonism. However, in contrast to Bakich et al (1984) and Pihan et al (1985) who observed cysteamine-HCl induced contractions of the rat duodenum and guinea-pig ileum, studies by Krantis and coworkers (1987, 1989) showed that in the rat small intestine, the primary action of this ulcerogen was to induce concentration-dependent relaxations through enteric non-adrenergic, non-cholinergic (NANC) inhibitory motor neurons. Krantis and Krause (1989)

proposed that earlier studies of the mammalian intestine did not observe cysteamine-HCl induced relaxations due to the 'low tone' of the isolated tissue preparations. Data obtained from in vitro and in vivo studies show contradictory effects in respect to the actions of cysteamine-HCl on gastrointestinal motor events. This observation is not surprising since it has been previously demonstrated with other drugs (norepinephrine, morphine) that the in vitro situation does not necessarily correlate to in vivo conditions (Daniel, 1968).

Both human and animal studies demonstrate that dysmotility, in addition to secretory alterations has a potential role in the pathogenesis of duodenal ulceration. These studies suggest that hypermotility and abnormal contractile activity of the gastroduodenum may interfere with normal acid clearing and neutralization mechanisms, preventing proper mixing and neutralization of gastric acid and duodenal alkaline secretions. This action may result in a reduced pH of the proximal duodenum, predisposing this region to ulcer formation (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Szabo and Vattay, 1990). However, it still has not been determined if the cysteamine-HCl induced disruptions in motility play a role in the location of the ulcers within the proximal duodenum (Takeuchi et al, 1987).

1.10 Summary.

The cysteamine-HCl animal model for chemically inducing duodenal ulcers in rats has allowed a partial reconstruction of some of the pathogenetic factors involved in the development of duodenal ulcer disease, particularly in the initial or early stages which cannot be investigated in duodenal ulcer patients (Szabo, 1984). Studies using the cysteamine-HCl rat model to investigate duodenal ulcer disease, have demonstrated that both peripheral and/or central mechanisms are involved and that this disease is clearly a heterogeneous disorder, involving secretory, biochemical, hormonal, vascular, motor and histological changes within the gastroduodenum.

In 1984, Szabo proposed that there are at least three major pathogenetic factors involved in cysteamine-HCl induced duodenal ulceration. These factors are represented in Figure 2. The secretory, motor and mucosal defense alterations that occur during the development of duodenal ulceration have been proposed to be dependent upon the neuroendocrine status of the organism (Szabo, 1984).

Aggressive factors, such as pepsin and hypersecretion of gastric acid, and reduced mucosal defense mechanisms are clearly important and contribute to the development of duodenal ulcers. However, these factors alone do not result in duodenal ulceration and cannot account for the location of the lesions (Szabo, 1984; Takeuchi et al, 1987). Evidence suggests that neurotransmitters, including noradrenaline, dopamine, 5-HT, histamine and GABA, as well as hormonal

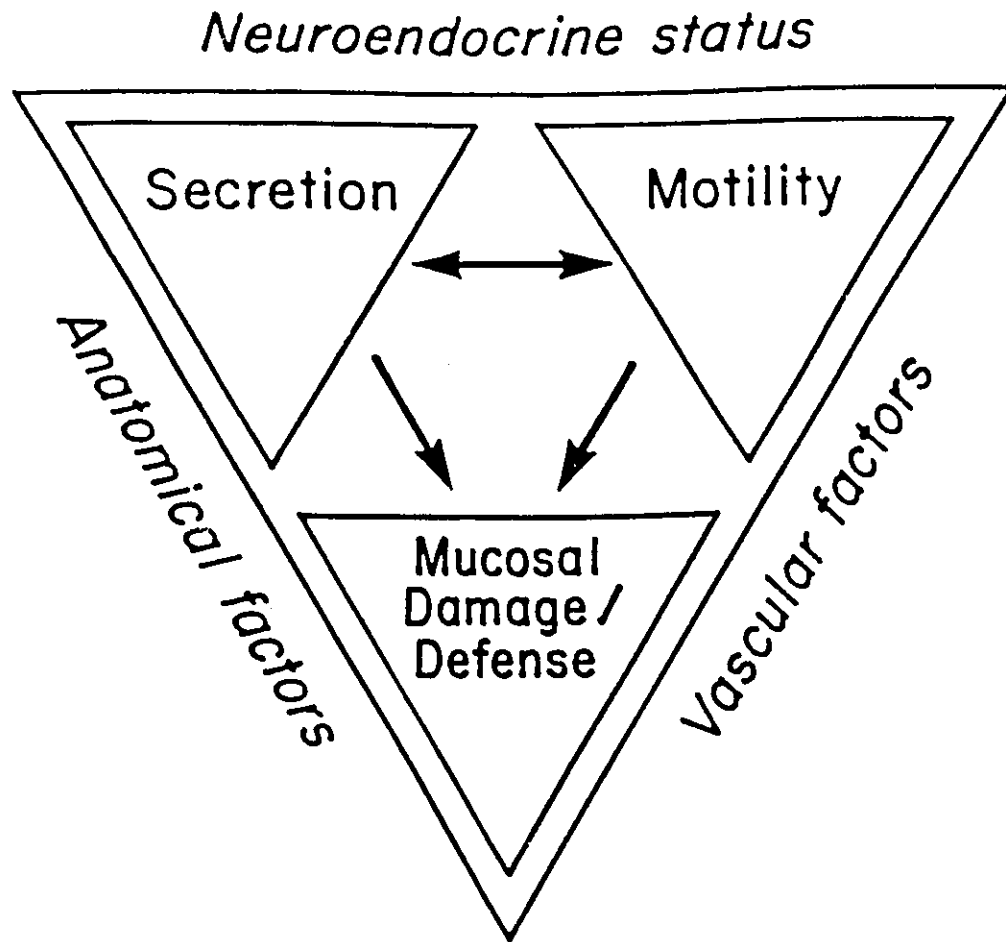


Figure 2. A schematic representation of the major pathogenetic factors associated with experimental duodenal ulceration. (Taken from Szabo, S. 1984. Laboratory Investigation. 51: 140).

(gastrin, somatostatin, secretin and epidermal growth factor), and vascular changes are also important factors which need to be considered in this disease.

Motility alterations of the gastroduodenum in cysteamine-HCl treated rats has recently been studied and correlated to the secretory, biochemical and functional changes associated with duodenal ulceration. These studies (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989) have demonstrated cysteamine-HCl induced duodenal dysmotility and proposed that motility alterations of the gastroduodenum may disrupt normal acid-base neutralization and proper mixing, resulting in the proximal duodenal mucosa being exposed to excess acid and thus, eventual ulcer formation. Moreover, in addition to anatomical factors and vascular changes, altered motor activity may help to contribute to the localization of duodenal ulcers within the proximal duodenum (Szabo, 1984).

No single factor is responsible for the formation of duodenal ulcers. The acid load presented to the duodenal bulb is clearly important to the disease process, and is dependent on both acid secretion and gastroduodenal motility. Thus, motility has important implications in the development of duodenal ulcers. Indeed, each factor within the known triad of ulcerogenic factors is important, and together, these factors contribute to the overall pathogenesis of this disorder.

1.11 General Aims.

A relationship appears to exist between gastroduodenal motility and the onset and development of duodenal ulceration. Previous in vivo studies have provided convincing evidence that gastrointestinal motility is disrupted prior to secretory, functional and biochemical changes and the macroscopic formation of the ulcer (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). In addition, in vitro studies have demonstrated that cysteamine-HCl has both neural and myogenic actions when applied to isolated stomach and small intestinal preparations of the rat and guinea-pig (Bakich et al, 1984; Pihan et al, 1985; Krantis, 1987; Krantis and Krause, 1989).

The overall aim of this project was to investigate potential disruption of intrinsic neural pathways involved in propulsive gastroduodenal motility, and to determine the importance of this disruption in the pathogenesis of duodenal ulceration.

This was studied using in vivo pharmacologic and physiologic experiments. Pharmacological experiments employed the cysteamine-HCl rat model for duodenal ulcers, and were designed to investigate the effects of stimulation of the peripheral GABAergic system, including enteric GABA sites, on the development of duodenal ulcer. In conjunction with this study, a separate series of pharmacological experiments were undertaken to test the effectiveness of a new series of compounds, the 'lazaroids', as anti-ulcer drugs. Compounds tested for

their pharmacological actions on cysteamine-HCl induced duodenal ulcer were subsequently investigated for their effects on gastroduodenal motility.

Physiological experiments designed to examine motility patterns required the development of an in vivo motility recording technique and data analysis software. In many studies employing in vivo techniques for the recording of gastrointestinal motility, problems were associated with the recording devices, and very often, anaesthetized animals were used. Under these conditions, proper recording and analysis of motility patterns could not be undertaken. In the present study, motor activity of the gastroduodenum was recorded in conscious, unrestrained rats which most closely resembles the normal, physiological situation.

These studies have provided evidence that the peripheral GABAergic system plays a role in the development of experimental duodenal ulcers. Moreover, motility, in addition to acid secretion and mucosal defense mechanisms, appears to be important in duodenal ulceration and thus, correction of motor disturbances may represent a new approach to the treatment of this disorder.

The results of the pharmacological studies, using the cysteamine-HCl rat model, are presented in Chapter 2. The development of the in vivo recording method for gastroduodenal motility and the results obtained using this technique are described in Chapter 3. The final chapter of this thesis, Chapter 4, is a discussion of the results of Chapters 2 and 3.

CHAPTER 2

Duodenal ulceration is a poorly understood disease which appears to involve hypersecretion of gastric acid, impaired mucosal defence mechanisms and altered gastroduodenal motor activity (McIntosh et al, 1984; Takeuchi et al, 1987; Ohe et al, 1988). Studies investigating the pathogenesis of duodenal ulcer disease have been aided by the use of specific animal models, including the rat.

Although various pharmacological techniques have been employed to chemically induce duodenal ulcers in the rat (see Chapter 1 for review), the specificity of many of these models was poor, with lesion formation occurring to widespread regions of the intestine. Currently, the most effective and widely used model for chemically inducing duodenal ulcers is that involving cysteamine-HCl treated Sprague-Dawley rats (Selye and Szabo, 1973; Robert et al, 1974; Fujii and Ishii, 1975; Szabo et al, 1977; Szabo, 1978, 1984; Tanaka et al, 1986; Krantis and Nicholson, 1989; Tanaka et al, 1989; Pan et al, 1990; Tanaka et al, 1990; Krantis and McKay, 1991; Krantis et al, 1992).

A number of biochemical transmitter substances, including γ -aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), somatostatin and dopamine have been proposed to be affected during the development of duodenal ulcer (Szabo et al, 1979, 1980; Gershon and Erde, 1981; Szabo et al, 1982; McIntosh et al, 1984; Szabo, 1984). It has previously been shown by Szabo et al (1982), that centrally

(icv) administered GABA causes aggravation of cysteamine-HCl induced duodenal ulcer while GABA_A antagonists exert protective actions. These observations suggest that central GABAergic sites may be involved in the development of duodenal ulcer.

GABA and its receptors are also present to autonomic ganglia, where histochemical evidence has shown GABA-like immunoreactivity in guinea-pig prevertebral sympathetic ganglia (Hills et al, 1988). Hills et al (1988) also determined that the prevertebral ganglia received GABAergic innervation from neurons peripheral to the ganglia, and suggested that this functional GABAergic innervation may originate from GABA-containing neurons from the myenteric plexus of the gastrointestinal tract. Moreover, electrophysiological studies showed that the actions of GABA on sympathetic neurons were similar to the effects of GABA in the periphery and on central neurons (Hills et al, 1988).

In addition, GABA is now established as a neurotransmitter of the mammalian enteric nervous system, where it is present to interneurons distributed throughout the wall of the gastrointestinal tract (Krantis and Kerr, 1981; Jessen et al, 1983; Krantis and Harding, 1987; Davanger et al, 1987; Hills et al, 1987; Taniyama et al, 1987; Krantis and Clark, 1991; Krantis and Clark, 1991). In fact, Krantis and Clark (1991) recently demonstrated the presence of GABAergic nerves in the submucosa and mucosa layers of the rat colon, with a network of fibres underlying the base of the mucosal crypts. GABA does not have direct actions on the intestinal smooth muscle but rather, exerts its effects (excitatory or inhibitory)

at two pharmacologically distinct neural receptors, the GABA_A- and GABA_B-receptor sites (see Chapter 1, Section 1.6.5) (Krantis et al, 1980; Krantis and Kerr, 1981; Giotti et al, 1983; Maggi et al, 1984; Krantis and Harding, 1986).

In addition to its suggested role in regulating intestinal motility, there is now considerable evidence showing both central and peripheral GABAergic nerve mediated modulation of acid secretion (Levine et al, 1981; Harty and Franklin, 1983; Tanaka, 1985; Harty and Franklin, 1986; Blandizzi et al, 1988; Del Tacca et al, 1989).

Recently, GABA has been proposed to function not only as an enteric neurotransmitter, but also as a gastrointestinal hormone, since GABA_A-receptors have been identified not only in the muscle layers of the antrum and body, but also to gland cells in the rat gastric mucosa and upper small intestine (Jessen et al, 1988; Erdö et al, 1989; Erdö et al, 1990; Gilon et al, 1990; Harty et al, 1991). Jessen et al (1988) also provides evidence that these GABA_A-receptor containing mucosal endocrine glandular cells possess high affinity GABA uptake sites. Gastric mucosal GABA_A-receptors have been suggested to be involved in the anti-ulcer actions of GABA on ethanol induced gastric ulceration and this GABAergic cytoprotection has been proposed to be due to a GABA-induced secretion of gastric mucus into the stomach (Lloyd et al, 1986; Erdö et al, 1989).

There is now evidence that GABA has modulatory interactions with certain endocrine cells in the rat stomach. Specifically, Gilon et al (1990) have demonstrated that there is a subpopulation of somatostatin-like and other still

unidentified endocrine cells of the rat stomach which can uptake [³H]-GABA while gastrin-like cells cannot. Moreover, exogenously applied GABA has been shown to stimulate gastrin release while inhibiting somatostatin release through stimulation of antral post-ganglionic cholinergic neurons in isolated mucosal fragments of the rat antrum (Harty and Franklin, 1983, 1986; Guo et al, 1989).

GABA receptors have also been identified on 5-hydroxytryptamine (5-HT) containing intestinal enterochromaffin cells where GABA was recently shown to modulate the release of 5-HT through cholinergic mechanisms (Schwörer et al, 1989). These studies provide further evidence for a role of GABA in secretomotor activity.

It has been well-established that GABA and its enzymes of synthesis are localized to the β -cells of the islets of Langerhans of the rat and human pancreas, indicating the coexistence of GABA and insulin (Vincent et al, 1983; Okada, 1986; Gilon and Remacle, 1989; Sternini et al, 1992). In addition, Gilon and Remacle (1989) demonstrated uptake of GABA by the islets of Langerhans also occurred in a certain subpopulation of somatostatin D cells, suggesting an endocrine function for GABA.

These various motor and neuroendocrine actions of GABA suggest the potential involvement of this system in the pathogenesis of duodenal ulceration. In 1989, Krantis and Nicholson showed that the systemic administration of GABA aggravated cysteamine-HCl induced ulceration which could be reversed by the specific GABA_A-receptor antagonist bicuculline. Enteric GABA_A-receptors may be

involved in the development of cysteamine-HCl induced duodenal ulcers since systemically administered GABA has previously been shown to be unable to readily cross the blood-brain-barrier (Rapoport, 1976; Krantis, 1983).

If enteric GABAergic sites are involved in the development of duodenal ulcer, this raises the prospect of new avenues for the study of the pathogenesis of duodenal ulcer disease, and of potentially important sites for therapeutic approaches to this disease. The aim of this study was to determine the extent of involvement of GABAergic mechanisms in experimental duodenal ulceration. In particular, I sought to investigate the effects of various systemically administered GABAergic compounds on cysteamine-HCl induced duodenal ulceration.

2.1 GABA, GABA Antagonists and Duodenal Ulceration.

2.1.1 Methods.

Male Sprague-Dawley rats (220-470g) were used in all experiments. Animals were fasted in sawdust cages for 24 hours, with free access to tap water prior to the initiation of drug treatments. All rats were administered two intragastric bolus doses of cysteamine-HCl, two hours apart, each at a dose of 28mg/100g *per os* (po). Rats were then maintained on tap water containing 0.1% cysteamine-HCl for 24 hours. Designated groups of animals were subjected to a variety of

pharmacological pretreatments prior to the administration of cysteamine-HCl.

Group A consisted of rats treated with cysteamine-HCl alone. Drug treatment groups were:

Group B: amino-oxyacetic acid (AOAA, 2.5mg/100g sc to prevent GABA degradation) plus γ -aminobutyric acid (GABA, 10mg/100g sc);

Group C: AOAA (2.5mg/100g sc) and the GABA_A-receptor antagonist bicuculline (30 μ g/100g sc) plus GABA (10mg/100g sc);

Group D: as for group C but with bicuculline (30 μ g/100g sc) dissolved in a saline vehicle;

Group E: same as group D but with bicuculline given at 45 μ g/100g (sc);

Group F: AOAA (2.5mg/100g sc) and the GABA antagonist picrotoxinin (100 μ g/100g sc) plus GABA;

Group G: same as group F but with picrotoxinin administered at a dose of 150 μ g/100g (sc).

The time-courses used with the different treatments is given in the appropriate figure legends.

Rats were sacrificed 24 hours following the second injection of cysteamine-HCl, and subjected to both macroscopic and microscopic evaluation of the proximal duodenum. In each rat, the abdomen was opened and the serosal surface of the gastroduodenum examined for evidence of ulceration. The stomach and proximal duodenum were then cut open along their lateral aspect to allow inspection of the mucosal surfaces for lesion formation.

The presence or absence of duodenal lesions was used to calculate the incidence of duodenal ulcer. The number of ulcers observed for each treatment group allowed the profile of ulceration (0, 1 or 2 ulcers) to be examined. To determine the severity of the mucosal lesions, a lesion index (mm²) of ulceration was established, with each lesion measured along the central length and width of the damaged area. Another indicator of the severity of mucosal lesions was the depth (intensity) of transmural damage. This parameter was assessed microscopically in the following way. Tissue segments taken from the ulcer region of the duodenal wall were rinsed with 0.9% saline, pinned out flat onto foam blocks and immersed in 10% formalin for a minimum of 24 hours. The fixed tissues were then embedded in paraffin wax, and transverse paraffin sections (12µm in thickness) were cut from the lesioned area of the duodenum, mounted onto subbed glass slides and stained with Alcian Blue.

The transmural intensity of ulceration was evaluated on a scale of 0-3 (0=normal, 1=superficial erosion of the mucosa, 2=deep ulcer with obvious transmural necrosis and 3=perforated or penetrating ulcer) as previously described by Szabo et al (1978). The evaluation of tissue samples was done under randomized, blinded conditions.

2.1.2 Statistical Analysis.

All quantitative data are expressed as the mean \pm SEM (standard error of

the mean). Group means were compared using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) test or the Student's t-test for unpaired observations. Fisher's Exact Test was used to determine the statistical significance of ulcer incidence. A probability value of $p < 0.05$ was considered to be statistically significant.

2.1.3 Drugs Used.

All drugs except for bicuculline (Groups C, D and E) were directly dissolved in 0.9% saline. Two different preparations of bicuculline were used: **a)** dissolved in ethylacetate and ethanol (1:3) (Group C) or **b)** dissolved in ethylacetate and ethanol and then, diluted in 0.9% saline (Groups D and E). Amino-oxyacetic acid, γ -aminobutyric acid, (+) bicuculline and picrotoxinin were obtained from Sigma. Cysteamine-HCl was purchased from Aldrich.

2.1.4 Effects of Cysteamine-HCl treatment.

Rats ($n=13$) administered two doses of cysteamine-HCl (28mg/100g po) showed obvious, macroscopic signs of duodenal ulceration at 24 hours (Figures 3 and 4). Ulcers were observed on the anterior and/or posterior aspects of the proximal duodenal wall, and were usually located within 2cm distal to the pyloric sphincter. All other regions of the gastrointestinal tract were completely free of

Figure 3. The effects of GABA analogues on the a) incidence and b) profile of ulceration of cysteamine-HCl induced duodenal ulceration over 24 hours. One or more lesions per animal was counted as one incident. The different treatment groups are indicated in the abscissa. Cysteamine-HCl (CYST) was administered in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was administered 120 minutes prior to cysteamine-HCl. GABA (10mg/100g sc) was administered 30 minutes before cysteamine-HCl. Bicuculline (BIC), 30µg/100g sc (dissolved in ethylacetate and ethanol) or 30µg/100g sc and 45µg/100g sc (BIC-S, diluted in saline) was given 60 minutes before cysteamine-HCl. Picrotoxinin (PIC), 100µg/100g sc and 150µg/150g sc, were administered 60 minutes prior to the cysteamine-HCl treatment. Data is expressed in percentages. n represents the number of rats.

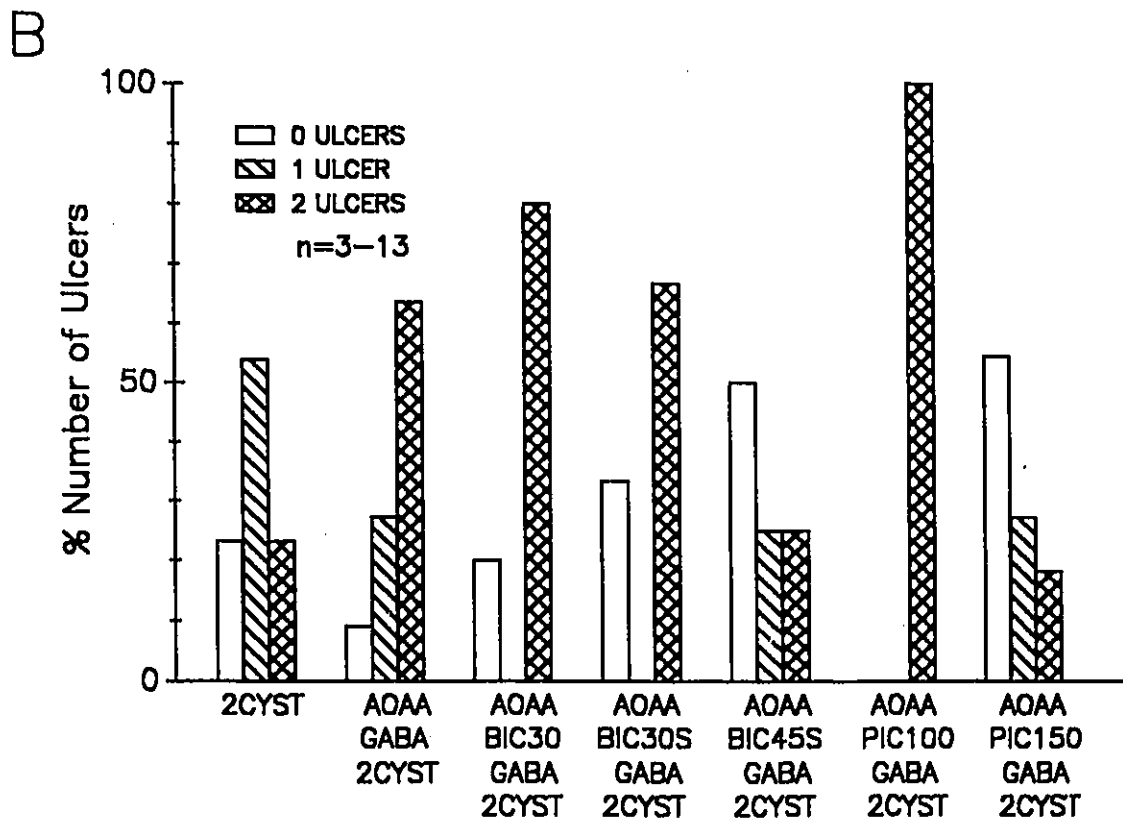
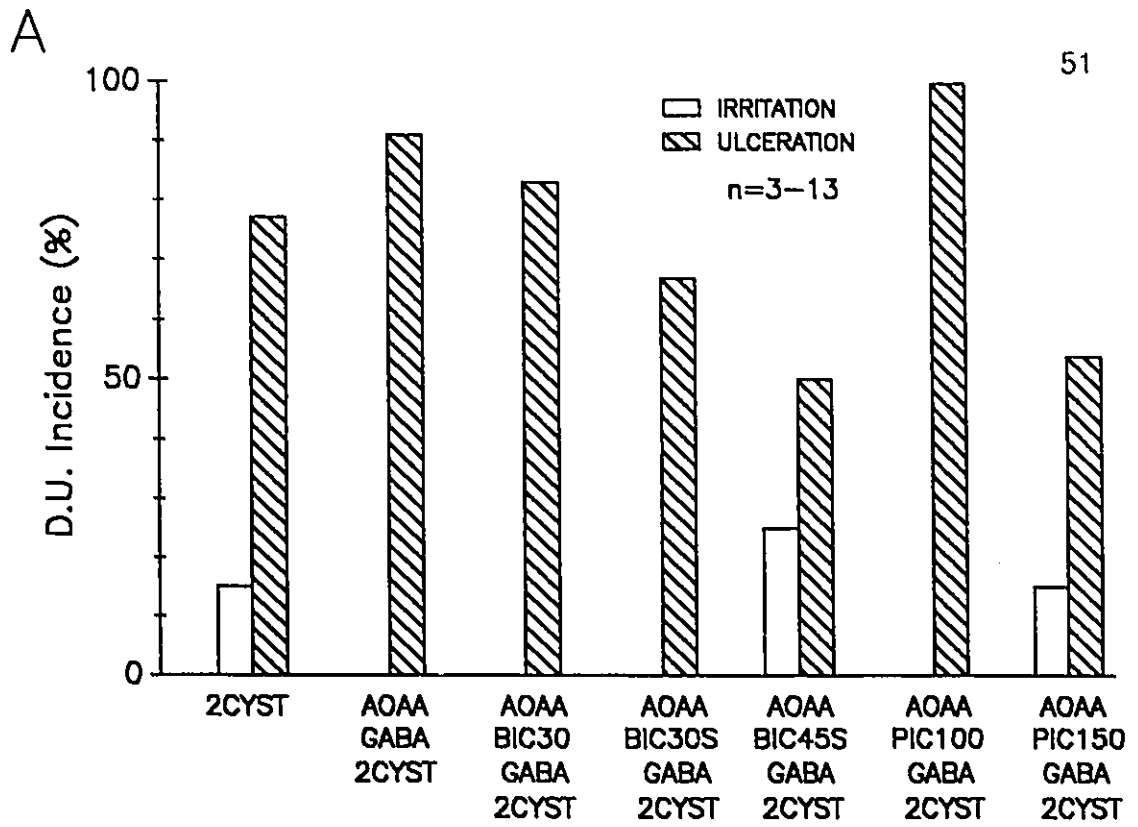
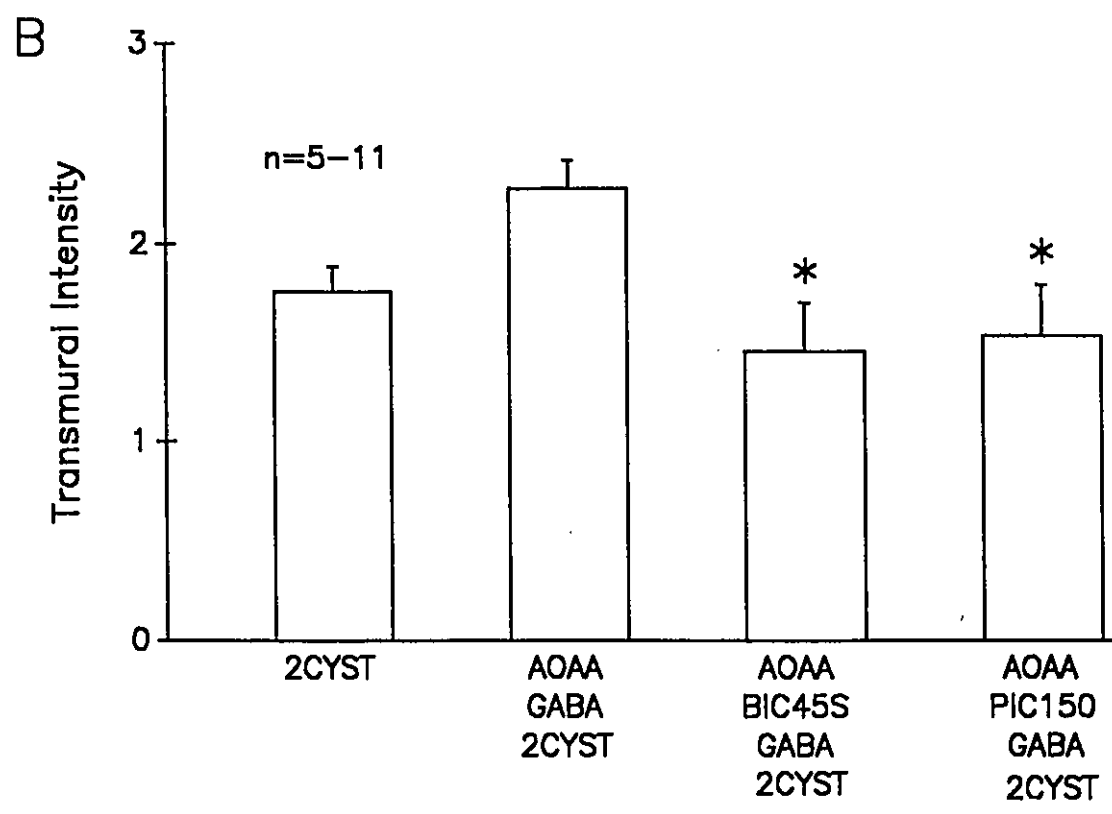
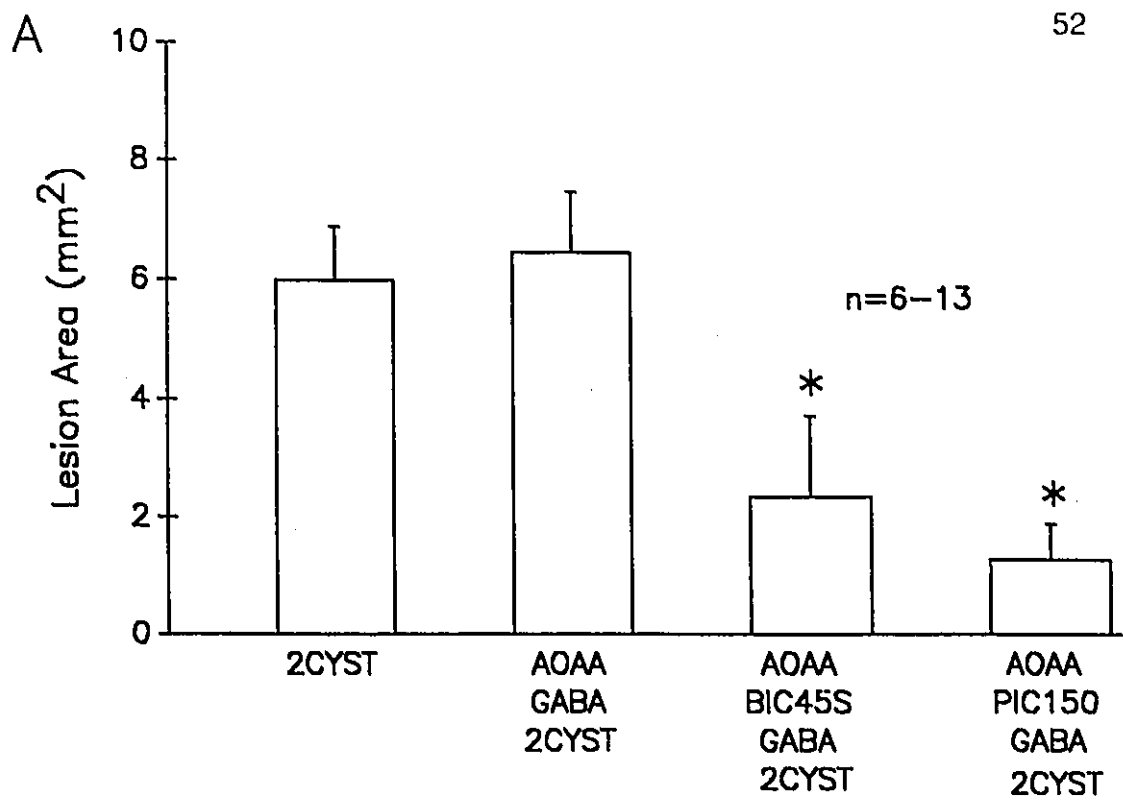


Figure 4. The effects of GABA analogues on the a) lesion area and b) transmural intensity of cysteamine-HCl induced ulceration over 24 hours. Individual treatment groups are represented in the abscissa. Cysteamine-HCl (2CYST) was administered in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was administered 120 minutes before cysteamine-HCl. GABA (10mg/100g sc) was given 30 minutes prior to cysteamine-HCl. Both bicuculline (BIC, 45µg/100g sc) and picrotoxinin (PIC, 150µg/100g sc) were administered 60 minutes before cysteamine-HCl. Data is expressed as the mean \pm SEM. Asterisks (*) denote statistical significance of $p < 0.05$ compared to the GABA plus AOAA pretreated rats. n represents the number of rats used.



mucosal lesions. Administration of the ulcerogen cysteamine-HCl in two boluses resulted in no mortality of the animals.

As shown in Figure 3a, duodenal ulceration occurred in 77% of cysteamine-HCl treated rats (n=13). Mucosal irritation, indicated by blanching and/or slight reddening of the luminal wall, was observed in 15% of rats. Only 8% of all rats treated with the ulcerogen showed no signs of mucosal disruption.

Solitary ulcers occurred in seven of the 13 treated rats while three rats displayed opposing ulcers (Figure 3b). There was no correlation in the pattern of ulceration and the body weight of the rats.

The average lesion area measured in these rats was $6.0 \pm 0.9\text{mm}^2$ (Figure 4a). As shown in Figure 4b, the transmural depth of the lesions was rated at 1.8 ± 0.1 out of 3, indicating that the induced ulceration was associated with significant transmural necrosis.

2.1.5 Effects of GABA on Duodenal Ulceration.

Animals pretreated with GABA (10mg/100g sc) were also administered amino-oxyacetic acid (AOAA, 2.5mg/100g sc) to prevent the degradation of systemically administered GABA. The subcutaneous administration of GABA, 60 minutes following AOAA pretreatment (Group B), caused an aggravation of cysteamine-HCl induced duodenal ulceration (Figures 3 and 4). Although the area of lesion damage was not significantly changed ($p>0.05$, Figure 4a) compared to

cysteamine-HCL treated rats (Group A), pretreatment with GABA plus AOAA (n=10) caused a 14% increase ($p>0.05$) in the incidence (Figure 3a), and a 30% increase ($p>0.05$) in the transmural depth of ulceration (Figure 4b). The profile of ulceration (ie. number of ulcers) was also clearly changed in animals pretreated with GABA plus AOAA, with 7 of 11 rats displaying opposing ulcers compared to only 3 of 13 cysteamine-HCl treated rats (Figure 3b).

2.1.6 Effects of GABA Antagonists on Duodenal Ulceration.

The aggravating effects of GABA on cysteamine-HCl induced ulcer were reversed by pretreatment with the specific GABA_A-receptor antagonist bicuculline (Figures 3 and 4). The actions of bicuculline were concentration-dependent, with maximal inhibition of the effects of GABA pretreatment occurring at a subcutaneous dose of 45 μ g/100g. However, the effectiveness of bicuculline was dependent on the vehicle used to dissolve the antagonist. Preparation of bicuculline in an ethylacetate/ethanol solution (Group C, n=6) was ineffective in reducing the aggravating effects of GABA on cysteamine-HCl induced duodenal ulceration (Figure 3). On testing alone, the ethylacetate/ethanol solution was found to cause transmural damage to the duodenal wall. Therefore, subsequent experiments used bicuculline dissolved in an ethylacetate/ethanol solution and then diluted with 0.9% saline to the appropriate dosage. In these animals (Groups D and E), bicuculline demonstrated anti-duodenal ulcer effects (Figures 3 and 4).

Bicuculline, at doses of 30 μ g/100g (Group D, n=3) and 45 μ g/100g (Group E, n=8), not only reversed the effects of GABA but in fact, reduced the incidence of ulceration (10% and 27% respectively, $p>0.05$) below the level established in rats treated with cysteamine-HCl (n=13) alone (Figure 3a). Bicuculline (45 μ g/100g) also shifted the ulcer profile, causing a 39% reduction ($p>0.05$) in the number of opposing ulcers observed compared to the GABA plus AOAA pretreated rats (Figure 3b). As shown in Figure 4a and b, the severity of ulceration induced by GABA was significantly reduced by bicuculline. Bicuculline (45 μ g/100g) reduced the lesion area of GABA aggravated ulceration by 64% ($p<0.05$), whereas the transmural intensity was reduced by 36% ($p<0.05$).

Picrotoxinin, an antagonist of the Cl⁻ ionophore associated with GABA_A-receptors, also blocked the aggravating effects of GABA in a concentration-dependent manner (Figures 3 and 4). At a dose of 100 μ g/100g (sc), picrotoxinin (Group F, n=4) was ineffective in preventing the GABA aggravation of cysteamine-HCl induced effects (Figure 3a and b). All rats treated with picrotoxinin at this dose showed evidence of opposing ulcers. However, picrotoxinin at a dose of 150 μ g/100g (Group G, n=13), resulted in a reduction of the GABA aggravated ulceration which was comparable to that observed with bicuculline (45 μ g/100g; Group E, n=8). At a dose of 150 μ g/100g, picrotoxinin reversed the GABA aggravated actions on ulcer incidence by 23% (Figure 3a). In fact, picrotoxinin treatment reduced the incidence of ulceration below the level established in cysteamine-HCl treated rats. Moreover, picrotoxinin (150 μ g/100g) reduced, by

45%, the number of animals with opposing ulcers (Figure 3b). The transmural intensity and lesion area of GABA aggravated ulcer were also significantly ($p < 0.05$) reduced (32% and 79% respectively) as shown in Figure 4a and b.

2.1.7 Summary.

The results obtained from these studies confirm and extend the initial findings of Krantis and Nicholson (1989). Using the cysteamine-HCl rat model, the administration of both GABAergic agonists and antagonists resulted in pharmacological manipulation of experimentally induced duodenal ulcer.

The subcutaneous administration of GABA, in the presence of AOAA, resulted in increased incidence of cysteamine-HCl induced ulceration with increased numbers of animals displaying double ulcers on the duodenal wall. This effect of GABA could be blocked, in a concentration-dependent manner, by both the GABA_A-receptor antagonist bicuculline, and by the GABA_A-receptor coupled Cl⁻ channel blocker picrotoxinin. These GABA antagonists not only reversed the GABA aggravated effects but also appeared to exert anti-ulcer actions, since the resulting incidence of ulceration was lower than the cysteamine-HCl control rats. However, the magnitude of the anti-ulcer actions of the GABA antagonists were not consistent for all of the parameters measured. Lesion area was reduced to the greatest extent by the GABA antagonists.

Taken together, these findings provide evidence which suggests that

peripheral, presumably enteric GABAergic mechanisms are involved in the pathogenesis of duodenal ulceration.

2.2 Baclofen and Duodenal Ulceration.

GABA and GABA_A-specific analogues (ie. muscimol) have been shown to stimulate gastric acid secretion (Levine et al, 1981; Szabo et al, 1982; Tsai et al, 1987; Blandizzi et al, 1988; Del Tacca et al, 1990) through central and peripheral GABA_A-receptor sites and these actions can be blocked by the specific GABA_A-receptor antagonist bicuculline. Baclofen, a GABA_B-receptor ligand, has been found to either stimulate (Goto and Debas, 1983; Goto et al, 1984; Pugh et al, 1985; Andrews and Wood, 1986; Blandizzi et al, 1988; Del Tacca et al, 1990; Takeuchi et al, 1990), inhibit (Lloyd et al, 1986; Del Tacca et al, 1990) or exert no effect (Tsai et al, 1987; Erdö et al, 1989; Del Tacca et al, 1990) on gastric acid secretion. These actions of GABA, GABA_A-related analogues and baclofen on gastric acid secretion are summarized in Table 2.

Subcutaneous administration of baclofen, a lipophilic compound, has been shown to stimulate gastric motility in the rat which has been attributed primarily to vagal activation of intramural postganglionic cholinergic excitatory nerves (Andrews and Wood, 1986; Takeuchi et al, 1990). In isolated gut bath preparations of the rat and guinea-pig intestine, baclofen blocked the excitatory cholinergic motor neurons by a prejunctional mechanism of action (Ong and Kerr, 1983; Giotti et al, 1983;

Table 2: Summary of the effects of GABA, GABA_A and GABA_B analogues, and GABA antagonists on gastric acid secretion and duodenal ulceration. ↑ = increased; ↓ = decreased and ↔ = no effect.

		GASTRIC ACID SECRETION	DUODENAL ULCERATION
GABA (no AOAA)	icv	↑ ↓	↑
	sc		↑
	antrum	↑	
	po	↑	
GABA and AOAA	sc	↑	↑
MUSCIMOL	icv	↑	
	iv	↔ ↑	↑
	sc	↑	
	antrum	↑	
THIP	ip	↓	
γ-VINYLGABA	sc		↑
BICUCULLINE	icv	↓ or ↔	
	sc		↓
PICROTOXININ	sc		↓
PICROTOXIN	sc		↓
BACLOFEN	icv	↑ ↓	
	iv	↑ ↔	
	sc	↑ ↓(ip)	↓
	antrum	↔	

Krantis and Harding, 1987). Furthermore, in the isolated guinea-pig distal colon, the antagonism of GABA_A-receptors and tachyphylaxis to baclofen resulted in a slowing of pellet expulsion which was similar to that observed with GABA tachyphylaxis alone. This is evidence that not only GABA_A- but also GABA_B-receptor sites may be involved in the control of motility (Ong and Kerr, 1983). However, *in vivo* studies show that baclofen stimulates duodenal motility in rats through central and peripheral muscarinic receptors (Fargeas et al, 1988).

It has recently been discovered (Krantis, 1990; Krantis and Clark, 1991; Krantis and Clark, 1991) that there is a rich GABAergic innervation of both the nerve and vascular plexuses in the rat submucosa. In addition, GABA_A-receptors have been identified on autonomic prevertebral sympathetic ganglia of guinea-pig (Hills et al, 1988); mucosal gland cells of rat stomach and upper small intestine (Jessen et al, 1988; Erdö et al, 1989; Erdö et al, 1990; Gilon et al, 1990; Harty et al, 1991) and in the rat and human pancreas (Vincent et al, 1983; Gilon and Remacle, 1989; Sternini et al, 1992). These studies demonstrate that the enteric GABAergic system is considerably more extensive than what was originally believed.

The aim of this study was to further investigate the involvement of peripheral GABAergic mechanisms in duodenal ulcer. In particular, GABA_B-receptor related events were examined and compared to the GABA_A-receptor sites in the modulation of cysteamine-HCl induced duodenal ulcer.

2.2.1 Methods.

Male Sprague-Dawley rats (200-435g) were used in all experiments. The experimental protocol used in these studies was identical to Section 2.1.1.

All rats received 2 oral doses of cysteamine-HCl, two hours apart, each at a dose of 28mg/100g. Rats were then maintained on 0.1% cysteamine-HCl treated tap water for 24 hours. Selected groups of rats were subjected to a variety of pharmacological pretreatments before the oral administration of cysteamine-HCl.

Group A consisted of animals treated with cysteamine-HCl alone. Drug treatment groups included:

Group B: baclofen (400µg/100g sc);

Group C: the H₂-receptor antagonist cimetidine (1mg/100g po);

Group D: amino-oxyacetic acid (AOAA, 2.5mg/100g sc to prevent GABA degradation) plus GABA (10mg/100g sc);

Group E: AOAA (2.5mg/100g sc) and the GABA_B-receptor agonist baclofen (400µg/100g sc) plus GABA (10mg/100g sc);

Group F: AOAA (2.5mg/100g sc) and cimetidine (1mg/100g po) plus GABA (10mg/100g sc).

Cimetidine (H₂-receptor antagonist) is a commonly used therapy in the treatment of duodenal ulcer disease in humans. Therefore, cimetidine was used in this study for direct comparison of the effectiveness of baclofen on the experimentally induced ulcers. The time-courses for the drug protocols used in the

different treatment groups are given in the figure legends.

2.2.2 Statistical Analysis.

See section 2.1.2 of this chapter.

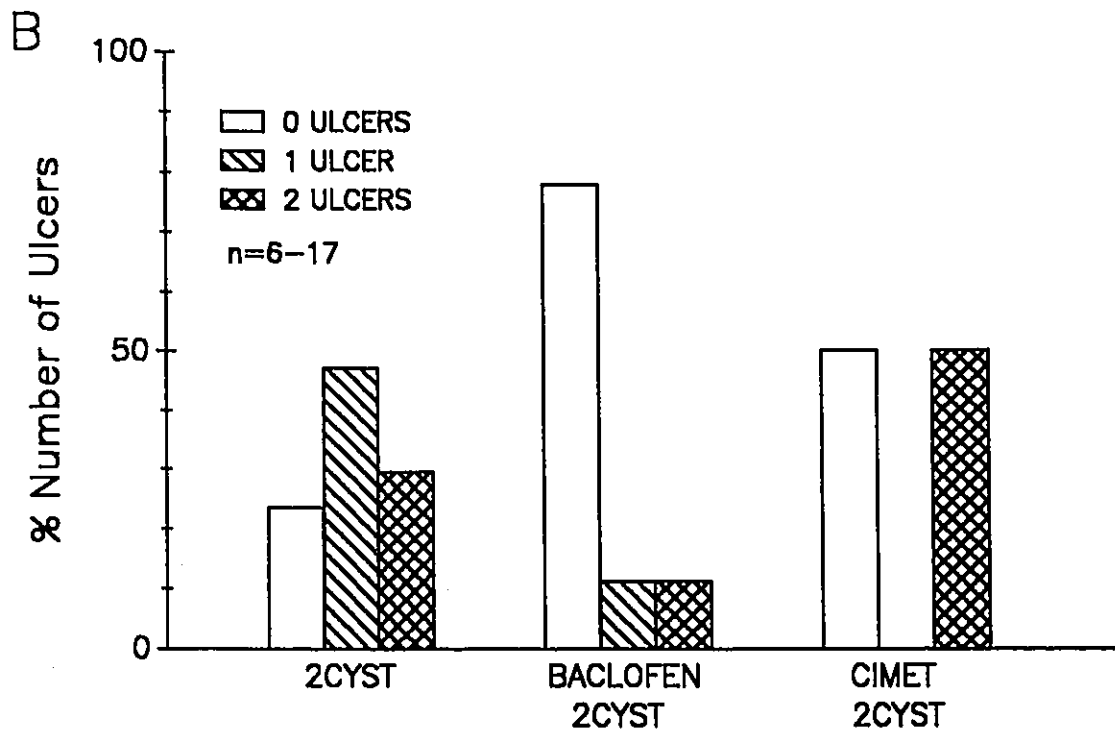
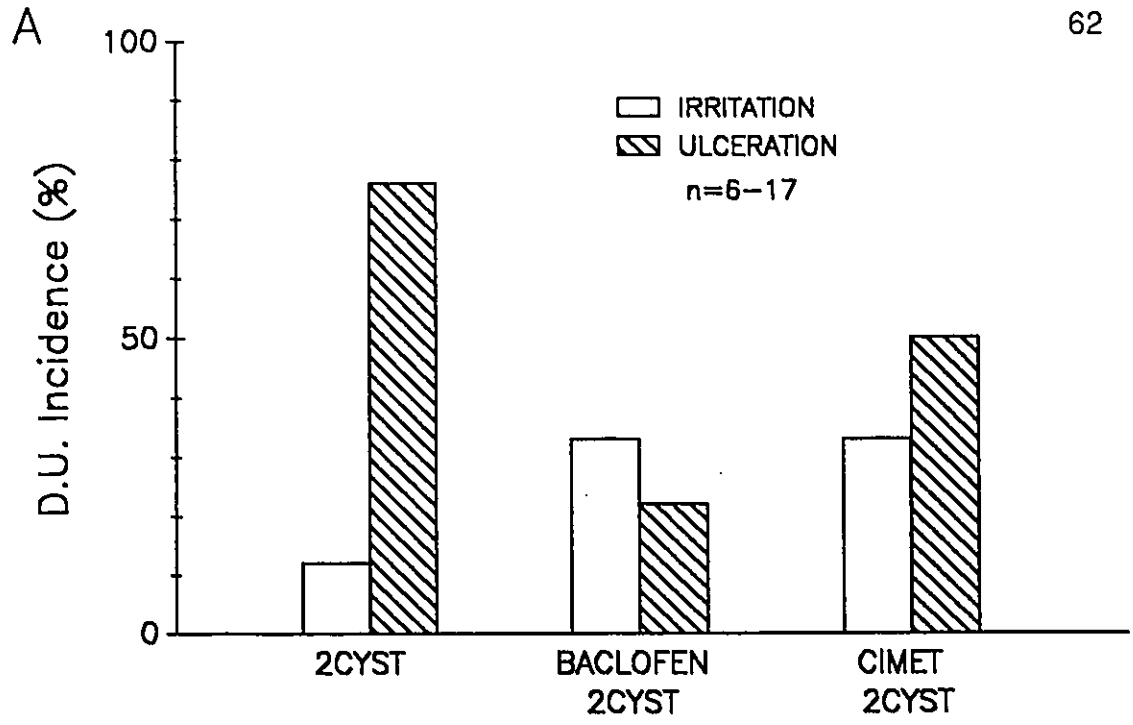
2.2.3 Chemicals Used.

Amino-oxyacetic acid (AOAA) and γ -aminobutyric acid (GABA) were obtained from Sigma. Cysteamine-HCl was obtained from Aldrich. Baclofen (Lioresal) was a gift from Ciba-Geigy. Cimetidine was provided as a gift from Smith, Kline and Beecham. All agents were dissolved in 0.9% saline.

2.2.4 Effects of Baclofen on Duodenal Ulcer.

Rats (n=17) treated with two doses of cysteamine-HCl (28mg/100g po) showed clear evidence of ulceration at 24 hours, with lesions localized to either the anterior and/or posterior aspects of the proximal duodenal wall. The incidence of ulceration in these animals was 76% (Figure 5a). Irritation of the mucosa only was observed in 12% of cysteamine-HCl treated rats. The majority (n=8/17) of cysteamine-treated rats showed evidence of a single lesion while five rats displayed opposing ulceration of the duodenum (Figure 5b). The average lesion

Figure 5. The effects of baclofen and cimetidine on the a) incidence and b) profile of ulceration of cysteamine-HCl induced duodenal ulceration. One or more lesions per animal was counted as one incident. The different treatment groups are indicated in the abscissa. Cysteamine-HCl (2CYST) was administered in 2 doses of 28mg/100g po. Both baclofen (BAC, 400µg/100g sc) and cimetidine (CIMET, 1mg/100g po) were administered 60 minutes prior to the cysteamine-HCl treatment. Data is expressed in percentages. n represents the number of rats.

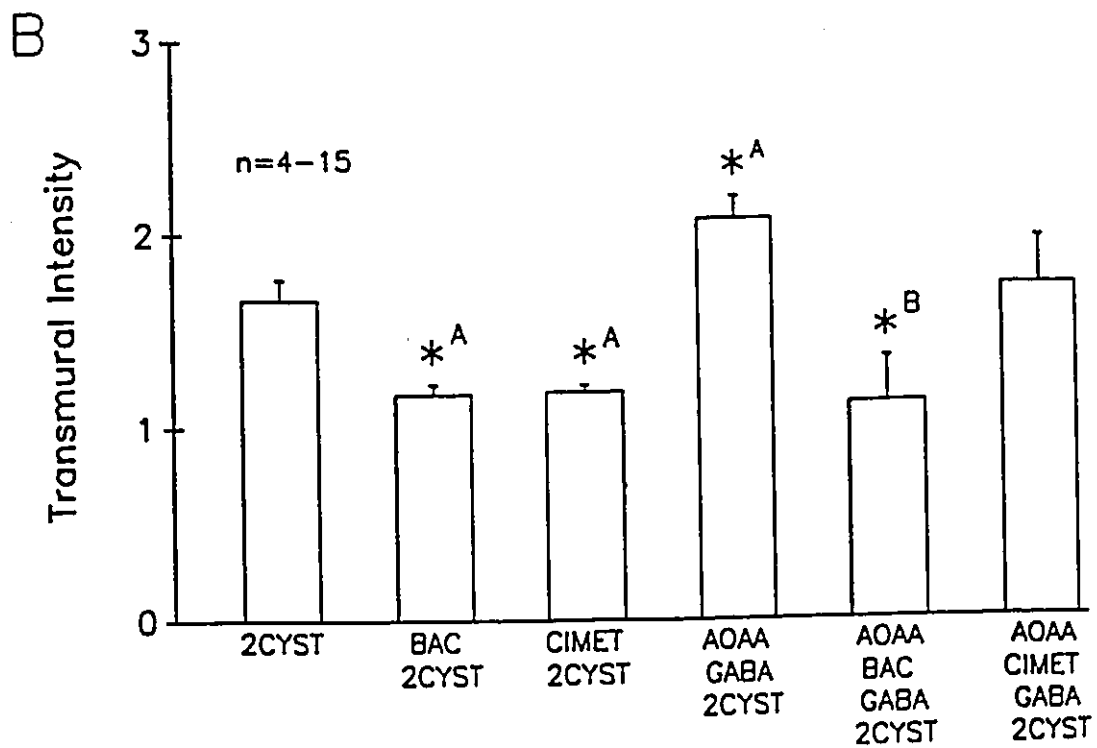
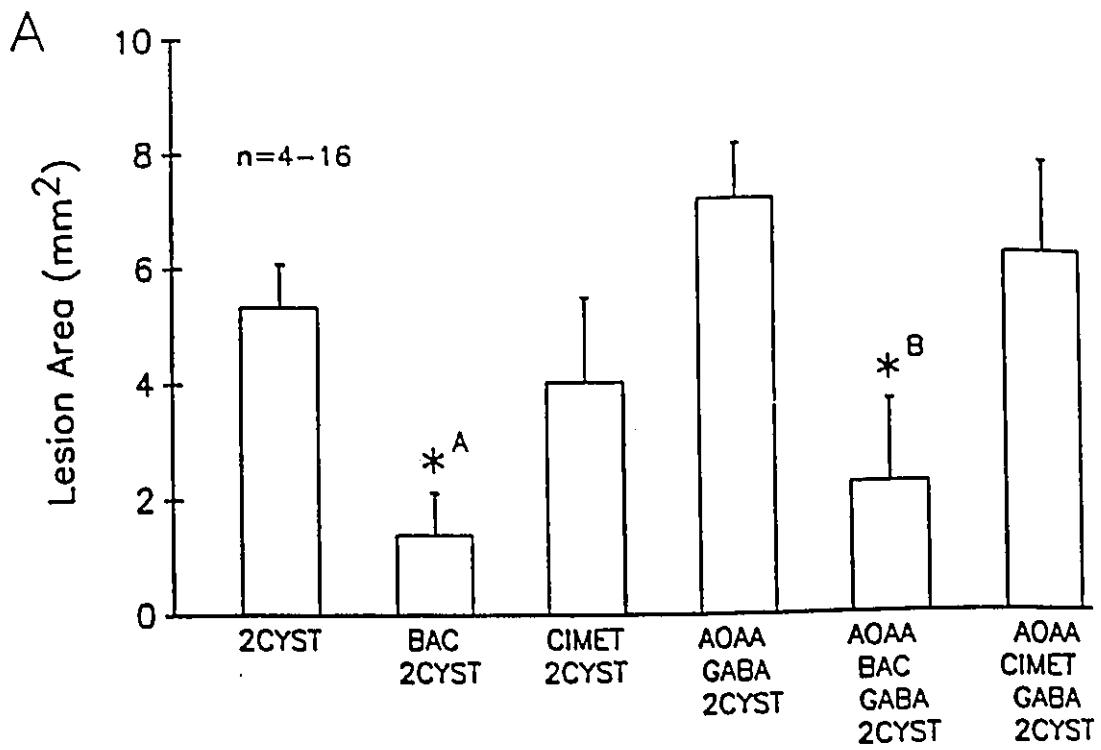


area following cysteamine-HCl treatment was $5.3 \pm 0.8\text{mm}^2$ (Figure 6a), and the transmural intensity of ulceration was rated at 1.7 ± 0.1 (Figure 6b).

Baclofen ($400\mu\text{g}/100\text{g sc}$), administered 60 minutes prior to cysteamine-HCl, caused a 54% reduction ($p>0.05$) in the incidence of cysteamine-HCl induced ulceration (Figure 5a). In fact, in four of nine rats, the duodenal wall was completely free of any lesion formation while in another 3 rats, only slight irritation of the mucosa was observed. Thus, the profile (ie. number of ulcers) of ulceration was also affected following the administration of baclofen, as shown in Figure 5b. Pretreatment with baclofen resulted in a significant reduction in lesion area (74%, $p<0.05$, Figure 6a) and transmural intensity (30%, $p<0.05$, Figure 6b) of ulceration compared to the control cysteamine-HCl treated rats.

Although cimetidine ($1\text{mg}/100\text{g po}$) reduced cysteamine-HCl induced ulceration by 26% ($n=6$), only three of these animals were free of duodenal lesions compared to seven of nine baclofen-treated rats as shown in Figure 5a and b. The remaining cimetidine-treated rats all showed evidence of opposing ulcers. Cimetidine treatment reduced the transmural depth of cysteamine-HCl induced ulceration by 29%, comparable to that observed with baclofen. However, the mean lesion area ($4.0 \pm 1.5\text{mm}^2$) for cimetidine treatment was not significantly different ($p>0.05$) from rats treated with cysteamine-HCl alone (Figure 6a and b).

Figure 6. The effects of GABA, baclofen and cimetidine on the a) lesion area and b) transmural intensity of cysteamine-HCl induced ulceration of 24 hours. Treatment groups are represented in the abscissa. Cysteamine-HCl (2CYST) was administered in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was administered 120 minutes before cysteamine-HCl. GABA (10mg/100g sc) was given 30 minutes prior to cysteamine-HCl. Both baclofen (BAC, 400µg/100g sc) and cimetidine (CIMET, 1mg/100g po) were administered 60 minutes before cysteamine-HCl. Data is expressed as the mean \pm SEM. Asterisks (*) denote statistical significance of $p < 0.05$ compared to cysteamine-HCl treated rats (*^A) or GABA plus AOAA pretreated rats (*^B). n represents the number of rats.



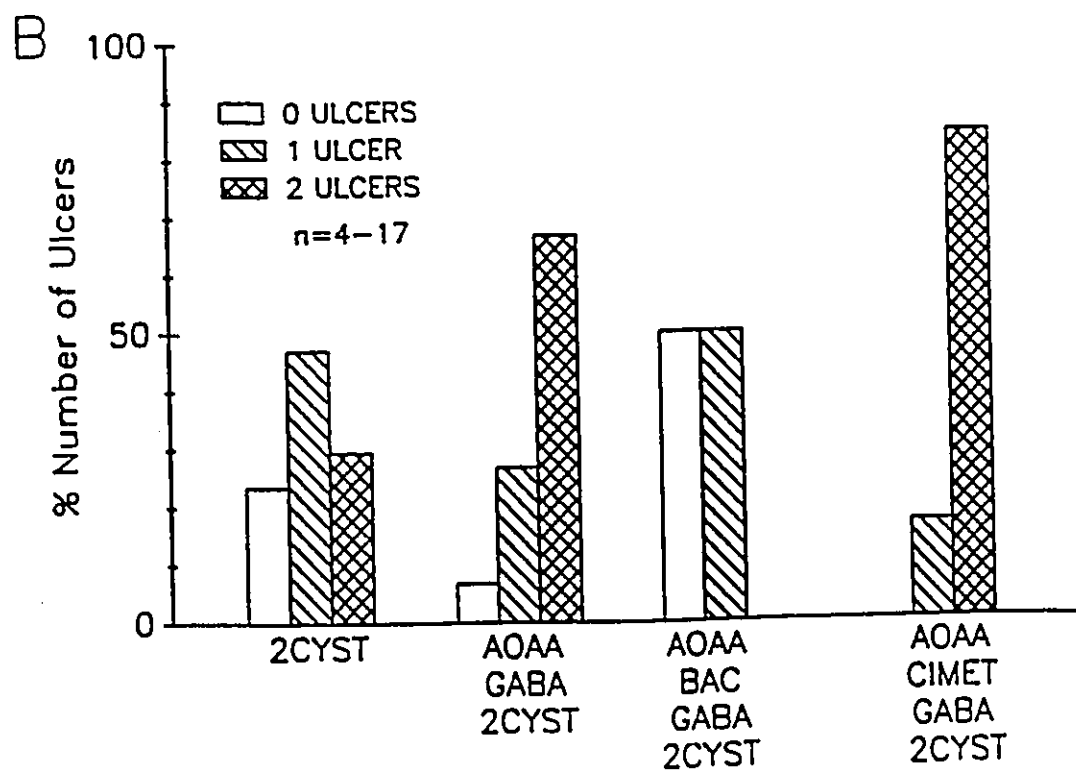
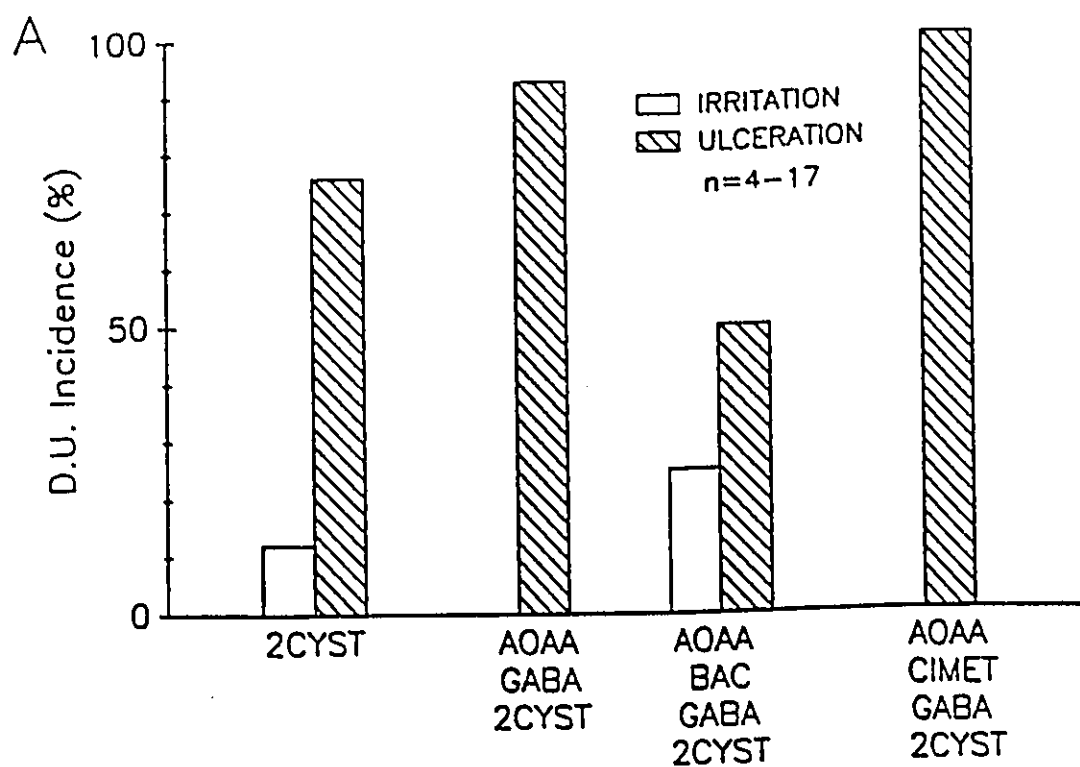
2.2.5 Effects of Baclofen on GABA-Aggravated Duodenal Ulceration.

As expected, treatment with GABA (10mg/100g sc) plus AOAA (2.5mg/100g, n=15), aggravated cysteamine-HCl induced duodenal ulceration (Figures 6 and 7). GABA increased the incidence of ulcer by 17% ($p>0.05$), with ten of these rats showing double ulcer formation compared to only five of seventeen cysteamine-HCl treated animals (Figure 7a and b). Although the mean lesion area was not significantly changed ($7.2 \pm 1.0\text{mm}^2$, $p>0.05$), the transmural depth of ulceration was significantly increased (25%, $p<0.05$, Figure 6a and b) in GABA-pretreated rats.

Pretreatment with baclofen ($400\mu\text{g}/100\text{g}$ sc) reversed the aggravating actions of GABA on all measured parameters of cysteamine-HCl induced duodenal ulcer (Figures 6 and 7). Baclofen (n=4) was not only protective against the aggravating actions of GABA, but also displayed anti-ulcer effects, since baclofen reduced the incidence of ulceration below that of the cysteamine-HCl treated rats by 26% ($p>0.05$, Figure 7a). In addition, animals pretreated with baclofen displayed no signs of opposing ulcer formation, with 50% of the rats being completely free of lesions (Figure 7b). Baclofen also caused significant reductions in the lesion area (69%, $p<0.05$, Figure 6a) and transmural intensity (46%, $p<0.05$, Figure 6b) of the GABA-cysteamine-HCl induced ulceration.

Cimetidine (1 mg/100g po) was ineffective in blocking the aggravating effects of GABA (Figures 6 and 7). All cimetidine pretreated rats (n=6) showed evidence

Figure 7. The effects of baclofen and cimetidine on the a) incidence and b) profile of ulceration of GABA-aggravated cysteamine-HCl induced duodenal ulceration. One or more lesions per animal was counted as one incident. The various treatment groups are indicated in the abscissa. Cysteamine-HCl (2CYST) was administered in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was given 120 minutes prior to cysteamine-HCl. GABA (10mg/100g sc) was administered 30 minutes before cysteamine-HCl. Both baclofen (BAC, 400µg/100g sc) and cimetidine (CIMET, 1mg/100g po) were administered 60 minutes before cysteamine-HCl. Data is expressed in percentages. n represents the number of rats.



of ulceration, with 83% displaying opposing ulcer formation on the duodenal wall (Figures 7a and b). In addition, pretreatment with cimetidine caused no significant ($p>0.05$) change in the lesion area or the transmural intensity of the GABA aggravated ulceration (Figure 6a and b).

2.2.6 Summary.

The results obtained from these studies support the proposal that peripheral GABAergic mechanisms can modulate cysteamine-HCl induced duodenal ulceration. In contrast to the aggravating effects of GABA, the peripheral administration of baclofen resulted in substantial reductions of all measured parameters of the induced ulceration. Moreover, baclofen not only reversed the aggravating effects of GABA, but reduced the ulceration below that of rats treated with cysteamine-HCl alone. In addition, baclofen was more effective than the H₂-receptor antagonist cimetidine.

This study provides evidence for the first time that not only enteric GABA_A-receptor sites but also enteric GABA_B-receptor sites are involved in the modulation of cysteamine-HCl induced ulceration. However, these peripheral GABAergic sites mediate opposite effects, with stimulation of GABA_A-receptors causing aggravation while stimulation of GABA_B-receptor sites caused an amelioration of the cysteamine-HCl induced ulceration.

Although baclofen has been widely used as an anti-spastic compound (Olpe

et al, 1978), it appears to have powerful, anti-ulcerogenic properties. This observation suggests that GABA_B-receptor sites may be potentially important in the development of new drug therapies for the treatment of duodenal ulcer disease.

2.3 U74500A and Duodenal Ulceration.

Previous studies in the laboratory of Dr. A. Krantis have examined the actions of a new compound from Upjohn, known as U74500A, on ethanol-induced gastric ulceration. This compound U74500A (21-(4-(3,6-bis(diethylamino)-2-pyridinyl)-1-piperazinyl)-16 α -methyl-pregna-1,4,9(11)triene-3,20-dione, hydrochloride) belongs to a novel series of 21-aminosteroids which are devoid of glucocorticoid or other classical steroidal properties (Braugher et al, 1988). Collectively termed the 'Lazaroids', these compounds have excellent therapeutic properties, reducing head/spinal cord trauma, and focal/global cerebral ischemia in experimental tissue injury models (Anderson et al, 1988; Hall et al, 1988). It appears that the lazarooids have a variety of characteristics that contribute to their therapeutic properties. They have been shown to be among the most potent inhibitors of lipid peroxidation (Braugher et al, 1989; Braugher and Pregonzer, 1989; Hall and Pazara, 1989). Furthermore, the class of 21-aminosteroids represented by the lazarooid compound U74500A not only catalyzes the decomposition of lipid hydroxyperoxides to peroxy and alkoxy radicals, but also binds ferrous iron which facilitates lipid-radical chain reactions through the

formation of oxygen radicals (Braugher et al, 1988, 1989; Hall and Pazara, 1989). These iron-chelating properties of U74500A enhances the anti-lipid peroxidation actions of this drug. In addition, the lazaroids are highly lipophilic compounds which enables them to partition into cell membranes and phospholipid environments to exert stabilizing effects (Braugher et al, 1988, 1989).

Due to the realization of the importance of oxygen radical and lipid peroxidation reactions to disease processes, increasing interest has been expressed in the lazaroid compounds beyond their intended central nervous system (CNS) use. These include treatment of renal and bowel ischemia (Braugher et al, 1989). Since the lazaroids have been shown to improve central neurological functions, these compounds may have the potential to correct enteric motor disturbances caused by the cytotoxic actions of cysteamine-HCl. Pharmacological studies of the mammalian intestine supports this proposal. In vitro studies of the rat intestine have shown that cysteamine-HCl not only stimulates the intrinsic, non-adrenergic non-cholinergic (NANC) inhibitory motor neurons, but also inhibits stimulation of the NANC inhibitory neurons by nicotinic agonists or by GABA (Krantis, 1987; Krantis and Krause, 1989). Krantis (1987) showed that these actions of cysteamine-HCl were not due to the generalized inhibition of the intrinsic NANC neurons or to the inability of the muscle to relax. Moreover, it was proposed by Krantis (1987) that the inhibition of the transmitter-induced relaxations by cysteamine-HCl resulted through interaction of this agent with receptor sites which are distinct from the operational transmitter receptor sites. It was also

speculated that cysteamine-HCl may disrupt cellular events associated with GABA stimulation of these inhibitory NANC motor neurons, possibly involving toxic actions.

In the cysteamine-HCl rat model, microscopic morphological changes in the duodenal mucosa strongly resembles lesions caused by ischemia (Szabo, 1984). Based on the potential therapeutic properties of U74500A, the initial studies examining the effects of U74500A on gastric ulceration were extended to cysteamine-HCl induced duodenal ulceration. Currently, clinical treatment of duodenal ulcer disease is through acid inhibition, using H₂-receptor antagonists such as cimetidine. However, acid secretion is not observed in more than one-third of duodenal ulcer patients (Koelz, 1986). Moreover, cessation of H₂-receptor blocker therapy is associated with a high incidence of ulcer relapse. A therapy is required which addresses all relevant pathogenetic factors of duodenal ulcer disease. **On this basis, U74500A was investigated to 1) evaluate the effectiveness of this compound as a potentially new, anti-duodenal ulcer drug and 2) determine whether U74500A interacts with enteric neural mechanisms associated with cysteamine-HCl actions.**

2.3.1 Methods.

2.3.1a in vivo studies.

Male Sprague-Dawley rats (220-470g) were used in all experiments. The experimental protocol is essentially the same as sections 2.1.1 and 2.2.1.

All rats were administered two intragastric bolus doses of cysteamine-HCl, two hours apart, each at a dose of 28mg/100g. Rats were then maintained on tap water containing 0.1% cysteamine-HCl for 24 hours. Selected groups of rats were then subjected to a variety of pharmacological treatments before the oral administration of the ulcerogen.

Group A consisted of rats treated with cysteamine-HCl alone. Drug treatment groups included:

Group B: U74500A (0.65mg/100g po);

Group C: Amino-oxyacetic acid (AOAA, 2.5mg/100g sc to prevent GABA degradation) plus γ -aminobutyric acid (GABA, 10mg/100g sc);

Group D: AOAA (2.5mg/100g sc) and U74500A (0.65mg/100g po) plus GABA (10mg/100g sc);

Group E: AOAA (2.5mg/100g sc) and U74500A (0.65mg/100g po) followed by a second dose 4 hours later plus GABA (10mg/100g sc);

Group F: AOAA (2.5mg/100g sc) and U74500A (1.3mg/100g po) plus GABA (10mg/100g sc).

The time-courses used for the various treatment groups (A-F) are indicated in the figure legends.

2.3.1b in vitro studies.

Male Sprague-Dawley rats (225-275g) were killed and exsanguinated, and segments of proximal duodenum and distal jejunum were quickly removed and placed into a modified Krebs's solution of the following composition (mmol/L): Na⁺, 151.0; K⁺, 4.6; Mg²⁺, 0.6; Ca²⁺, 2.8; Cl⁻, 134.9; HCO₃⁻, 24.9; H₂PO₄⁻, 1.3; SO₄⁻, 0.6; d-glucose, 7.7; gassed with a mixture of 95% O₂ and 5% CO₂, and maintained at 37°C, pH 7.4. The connective mesentery was carefully removed, and 2cm segments were positioned into separate 10ml organ baths for recording of mechanical activity in the longitudinal axis, with one end attached to the bottom of the glass organ bath and the other by a thread to an isometric strain gauge connected to a Grass polygraph (Model 7D). The individual tissue segments were then placed under a resting tension of 1.5g which was maintained throughout the experiment. Tissues were allowed to equilibrate in the organ bath for 60 minutes before the application of drugs.

Platinum ring electrodes were placed around segments of the small intestine in experiments in which the intramural nerves were being stimulated. Pulses of a 1 msec duration were delivered at a frequency of 5Hz for a 10 second stimulation period.

Artefactual alterations in the recordings sometimes occurred following the washout of the drugs, but these did not interfere with the responsiveness of the test tissue. However, the amplitude of the induced response was dependent upon the level of the tissue tone. Therefore, unless otherwise stated, U74500A and the other test drugs were applied, or retested, only when the basal tissue tone had recovered to within 90% of the resting level.

Concentration-response data for U74500A were derived using a non-cumulative regimen, with a minimum period of 10 minutes between drug challenges. The lowest concentration of the drug was administered first and left in the organ bath until the maximal response had been achieved. Concentration-response curves are expressed as a percent of the maximum response to the drug, independent of the concentration applied. When antagonist drugs were present in the bathing solution, the tissue segments were allowed to equilibrate for a minimum of 15 minutes or until the basal tone had recovered to within 90% of resting level before drug challenges were continued. The presented original recordings have been aligned to accurately show the relative level of tone for the individual tissue preparations at the time of recording. Concentrations of the drugs are given as the final concentration in the organ bath. Drug volumes used were never more than 1% of the bath volume.

2.3.2 Statistical Analysis.

in vivo studies: The statistical analysis used has been described in section 2.1.2 of this chapter.

in vitro studies: All data is expressed as the mean \pm SEM. The Student's t-test for unpaired samples was used to assess the significance ($p < 0.05$) of difference between mean values.

2.3.3 Chemicals Used.

All drugs used in the *in vivo* ulceration studies were dissolved in 0.9% saline. For the *in vitro* pharmacology studies, all drugs were dissolved in distilled water. U74500A (21-(4-3,6-bis(diethylamino-2-pyridinyl)-1-piperazinyl-16 α -methyl-pregna-1,4,9(11)triene-3,20-dione, hydrochloride) was provided by The Upjohn Company (Kalamazoo, Michigan). Amino-oxyacetic acid (AOAA), γ -aminobutyric acid (GABA), carbachol, 5-hydroxytryptamine (5HT) and 1,1-dimethyl-4-phenylpiperizinium iodide (DMPP) were purchased from Sigma. Cysteamine-HCl was obtained from Aldrich.

2.3.4 Effects of U74500A on Duodenal Ulcer.

Rats (n=27) treated with two oral doses of cysteamine-HCl (28mg/100g) displayed evidence of duodenal ulceration at 24 hours (Figures 8 and 9), with ulcers localized to the anterior and/or posterior aspects of the duodenal wall. The incidence of ulceration was 85%, while mucosal irritation only was observed in 11% of these rats (Figure 8a). Opposing ulceration was present in 17 cysteamine-HCl treated rats while seven showed evidence of solitary ulcer formation (Figure 8b). The average lesion area was $6.3 \pm 0.6\text{mm}^2$, and the intensity of these lesions was rated at 2.1 ± 0.1 following cysteamine-HCl treatment (Figure 9a and b).

Treatment of rats (n=4) with U74500A (0.65mg/100g) alone resulted in no macroscopic or microscopic evidence of duodenal ulceration.

U74500A (0.65mg/100g po), administered 60 minutes prior to the cysteamine-HCl treatment, caused significant reductions in the induced ulceration (Figures 8 and 9). Rats pretreated with U74500A (n=25) demonstrated a significant 49% ($p<0.05$) reduction in the cysteamine-HCl induced ulceration (Figure 8a). The profile (ie. number of ulcers) of ulceration was clearly changed in these rats (Figure 8b), with a 35% reduction ($p<0.05$) in the number of double lesions observed. Moreover, the majority (15/25) of these animals were completely free of lesions. Treatment with U74500A also significantly ($p<0.05$) reduced both the lesion area (53%, Figure 9a) and transmural intensity (31%, Figure 9b) of the induced ulceration.

Figure 8. The effects of the lazaroid U74500A and GABA on the a) incidence and b) profile of ulceration of cysteamine-HCl induced ulceration in rats over 24 hours. One or more lesions per animal was counted as one incident. The different treatment groups are indicated in the abscissa. Cysteamine-HCl (2CYST) was administered in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was given 120 minutes prior to cysteamine-HCl. GABA (10mg/100g sc) was injected 30 minutes before cysteamine-HCl. U74500A (0.65mg/100g po) was administered 60 minutes before cysteamine-HCl. Data is expressed in percentages. Asterisks (*) denote statistical significance ($p < 0.05$) compared to cysteamine-HCl treated rats (*^A) and GABA plus AOAA pretreated rats (*^B). n represents the number of rats used.

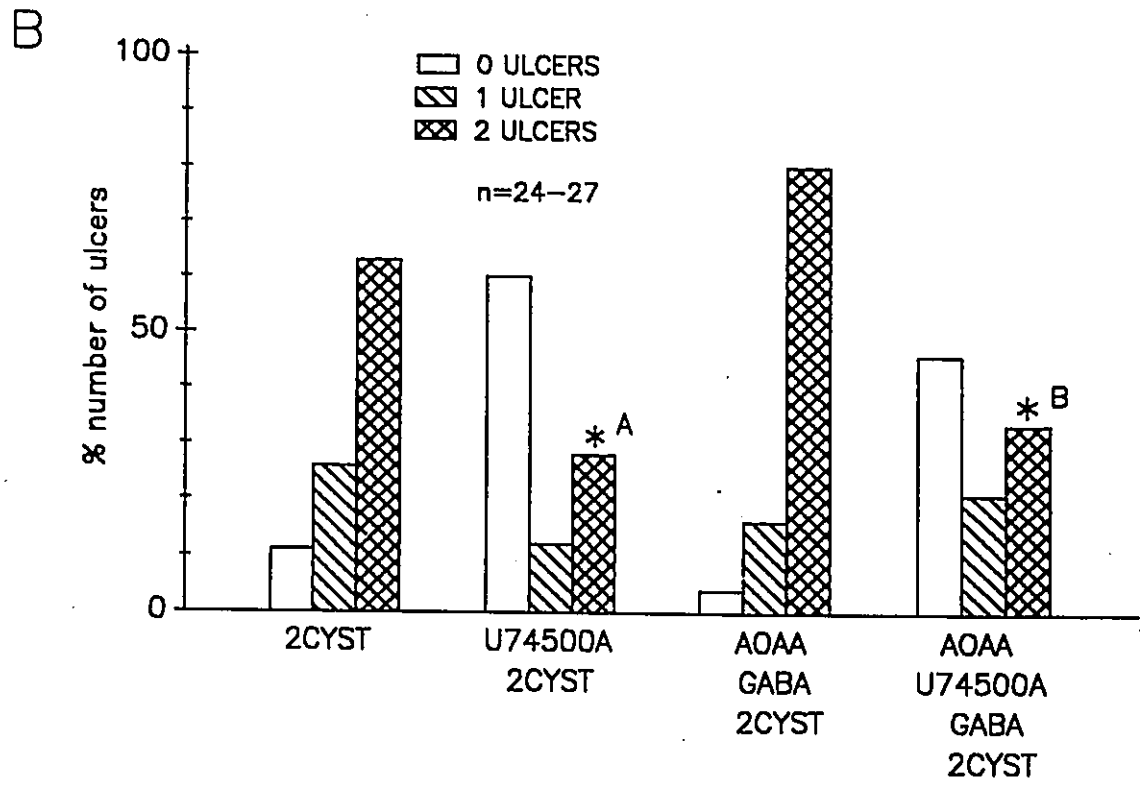
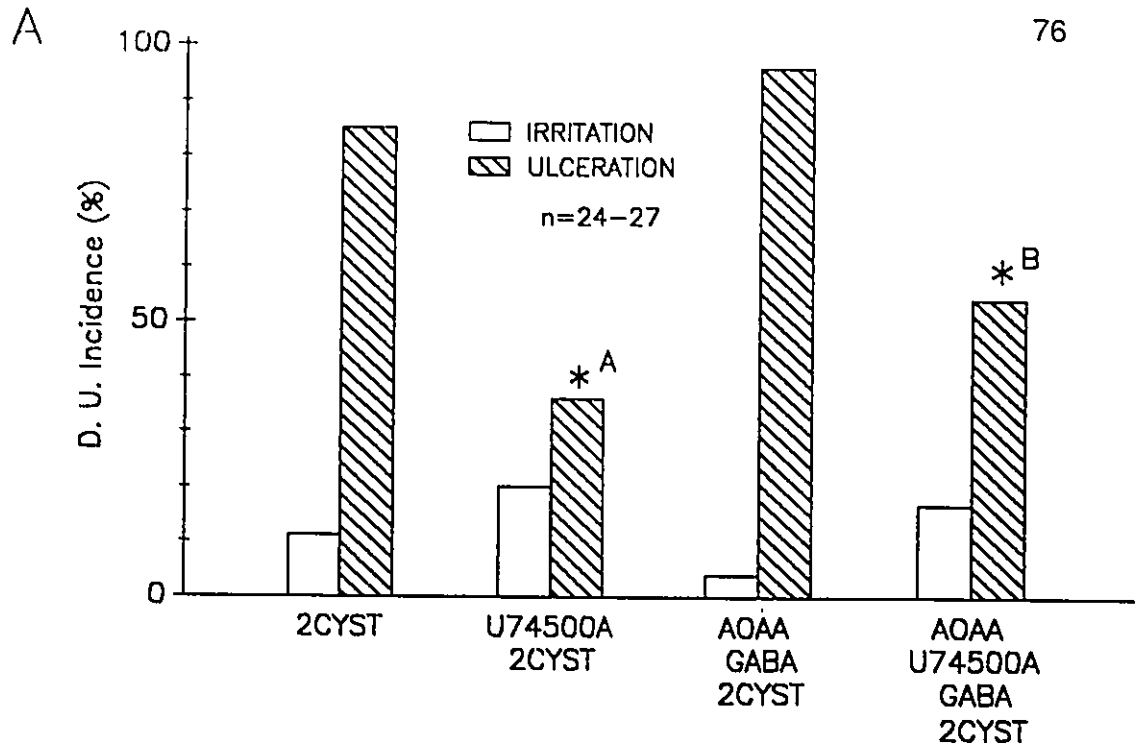
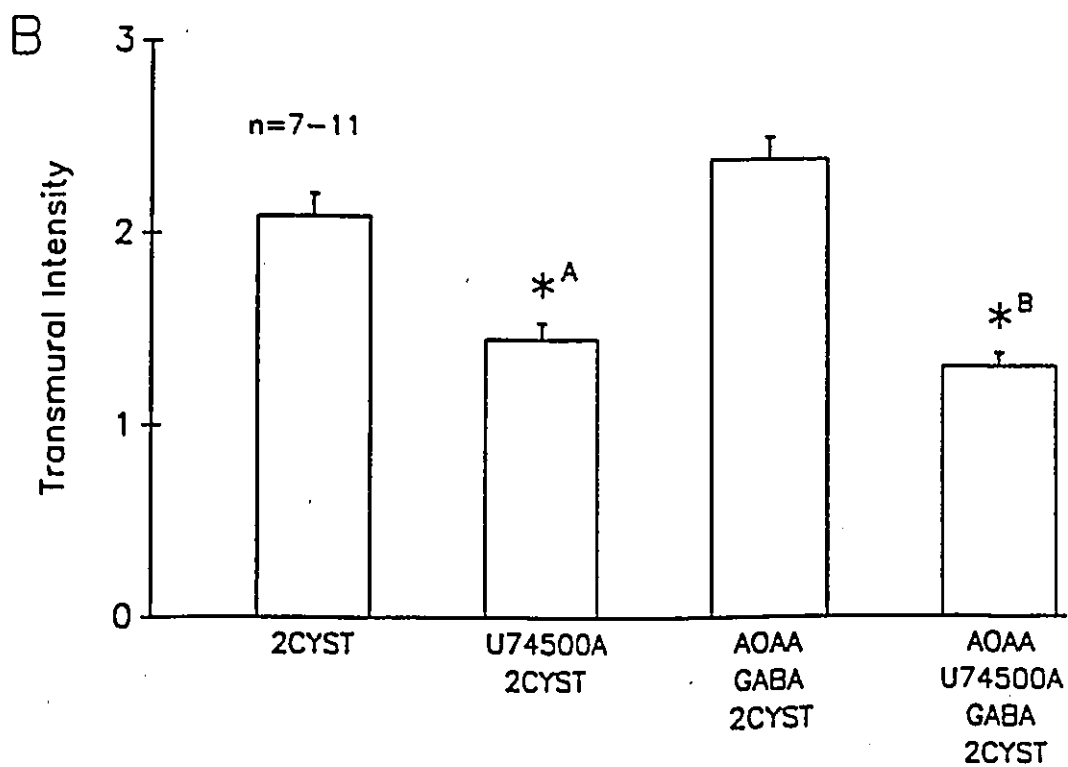
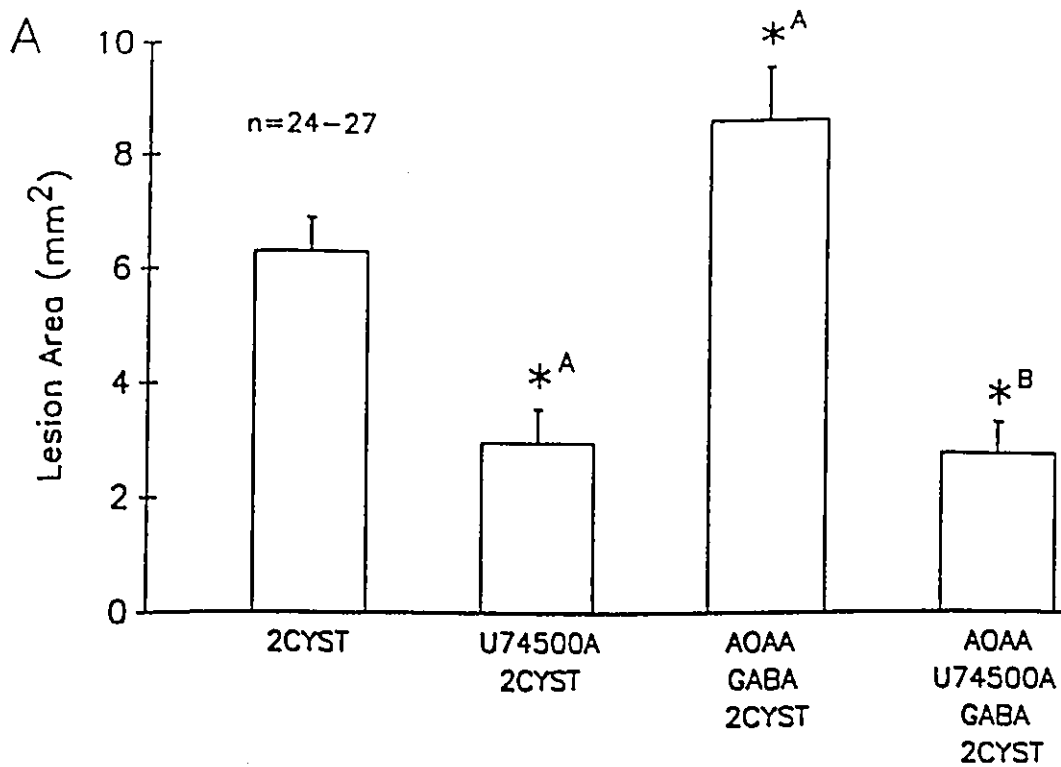


Figure 9. The effects of the lazaroid U74500A and GABA on the a) lesion area and b) transmural intensity of cysteamine-HCl induced ulceration over 24 hours. Treatment groups are shown in the abscissa. Cysteamine-HCl (2CYST) was given in 2 doses of 28mg/100g po. AOAA (2.5mg/100g sc) was administered 120 minutes before cysteamine-HCl. GABA (10mg/100g sc) was given 30 minutes prior to cysteamine-HCl. The lazaroid, U74500A (0.65mg/100g po), was administered 60 minutes before cysteamine-HCl. Data is expressed as the mean \pm SEM. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to cysteamine-HCl treated rats (*^A) or GABA plus AOAA pretreated rats (*^B). n represents the number of rats.



2.3.5 Effects of U74500A on GABA-Aggravated Duodenal Ulceration.

As expected, (see sections 2.1.5 and 2.2.5) GABA (10mg/100g sc) plus AOAA (2.5mg/100g sc, n=25) augmented the cysteamine-HCl induced ulceration (Figures 8 and 9). Opposing lesions were observed in 80% of these animals while 16% displayed evidence of a solitary ulcer. Although the transmural intensity of ulceration was not significantly ($p>0.05$) different from cysteamine-HCl treated rats, pretreatment with GABA significantly ($p<0.05$) increased (37%) the area of mucosal damage (Figure 9a and b).

The aggravating actions of GABA on the cysteamine-HCl induced ulceration were significantly reversed by U74500A (n=24), with the incidence of ulceration reduced by 42% ($p<0.05$), Figure 8a. U74500A not only exerted protective actions against the GABA aggravation, but also demonstrated anti-ulcer actions since the incidence of ulceration was reduced below that observed in cysteamine-HCl treated rats (n=27). Only 33% of U74500A pretreated rats displayed opposing ulceration compared to 80% of the GABA-aggravated rats (Figure 8b). Moreover, 46% of rats treated with U74500A were completely free of duodenal lesions. U74500A also induced significant reductions in both the lesion area (68%, $p<0.05$), and transmural depth of ulceration (45%, $p<0.05$) as presented in Figure 9a and b.

U74500A was also tested at a higher concentration of 1.3mg/100g po (Groups E and F, see Methods, section 2.3.1) against the GABA-aggravated

cysteamine-HCl induced ulceration. U74500A, administered in a single bolus (Group F, 1.3mg/100g po) or in two doses (0.65mg/100g po, 4 hours apart), significantly reversed ($p < 0.05$) the GABA-induced aggravation (Figures 10 and 11). The method of administration of the higher dose of U74500A did not change the effects of this drug on the incidence (Figure 10) or transmural intensity (Figure 11b) of ulceration. However, the administration of U74500A in two doses (each 0.65mg/100g po) was as effective as a single lower dose (0.65mg/100g po) in reducing ($p < 0.05$) the area of mucosal damage in GABA aggravated rats (Figure 11a). Thus, the higher doses of U74500A were not any more effective in reversing the GABA aggravation than a single, lower dose.

2.3.6 Effects of U74500A on the Small Intestine: *In Vitro* Studies.

U74500A was tested in organ bath preparations of isolated segments of the proximal duodenum and distal jejunum, to determine the pharmacology of its actions in the rat intestine. U74500A ($6.5 \times 10^{-6} \text{M}$ - $9.0 \times 10^{-5} \text{M}$) induced concentration-dependent contractions which were immediate in onset and maximal within 10 seconds, as shown in the typical recordings taken from the rat jejunum in Figure 12a. Concentrations of U74500A greater than $9.0 \times 10^{-5} \text{M}$ could not be tested due to the precipitation of the lazaroid in the organ bath. These concentration-dependent responses were transient, and recovery of the tissue tone to within 90% of the basal, resting level occurred within 90 seconds in the

Figure 10. The effects of U74500A (0.65mg/100g, 2x0.65mg/100g or 1.3mg/100g po) on the incidence of GABA-aggravated cysteamine-HCl induced ulceration. The different treatment groups are shown in the abscissa. Cysteamine-HCl (2CYST) was given in 2 doses of 28mg/100g po. U74500A (0.65mg/100g or 1.3mg/100g po) was administered in a single bolus, 60 minutes before cysteamine-HCl. U74500A (2x0.65mg/100g po) was given in two separate boluses, 4 hours apart, with the first bolus administered 60 minutes before cysteamine-HCl. Data is expressed in percentages. n represents the number of rats.

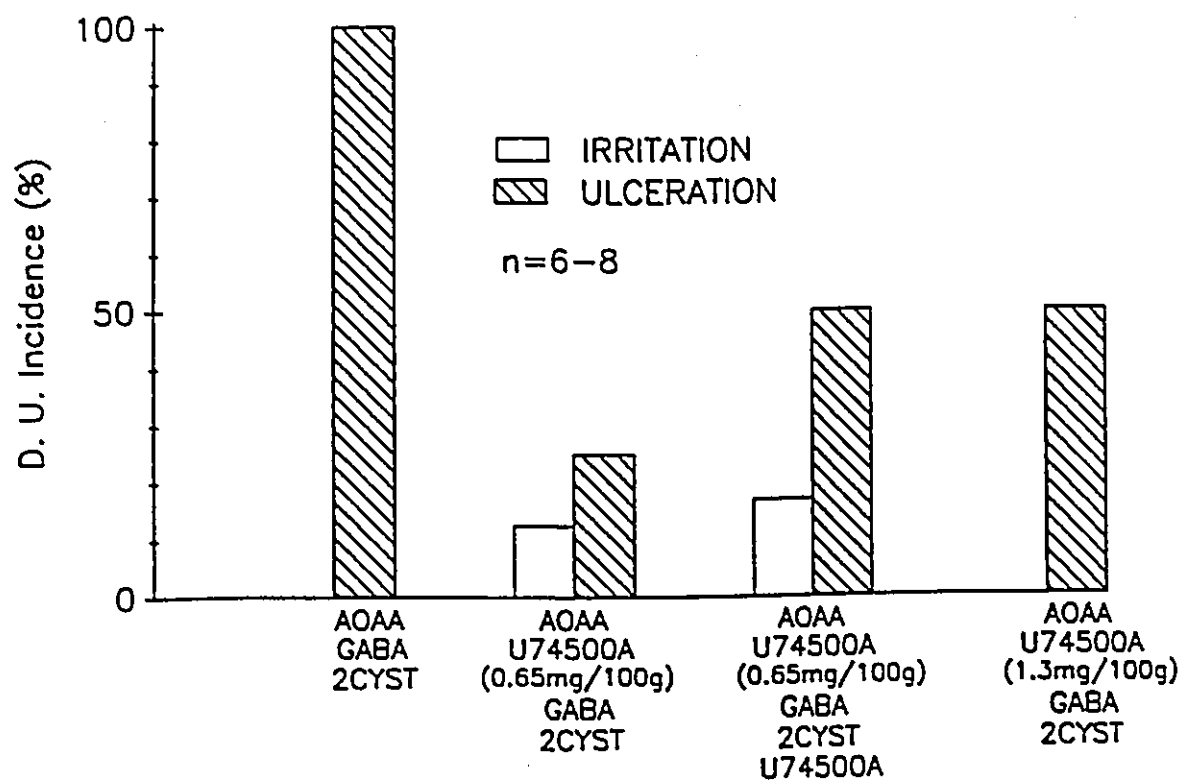


Figure 11. The effects of different doses of U74500A (0.65mg/100g, 2x0.65mg/100g or 1.3mg/100g po) on the a) lesion area and b) transmural intensity of GABA-aggravated cysteamine-HCl induced ulceration in rats over 24 hours. The various treatment groups are represented in the abscissa. Cysteamine-HCl (2CYST) was given in 2 doses of 28mg/100g po. U74500A, at single doses of either 0.65mg/100g or 1.3mg/100g po, was given 60 minutes before the ulcerogen. U74500A was also administered in 2 separate doses of 0.65mg/100g po each, with the first bolus given 60 minutes before cysteamine-HCl. Asterisks (*) denote statistical significance ($p < 0.05$) compared to the GABA plus AOAA pretreated rats. n represents the number of rats used.

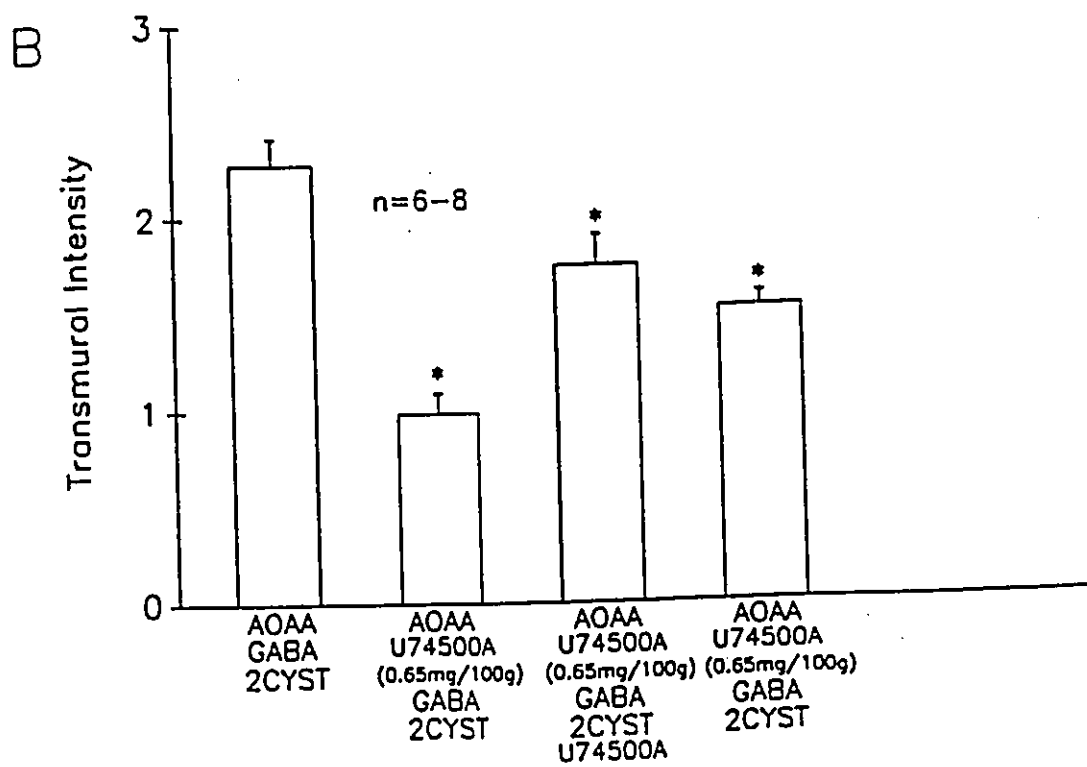
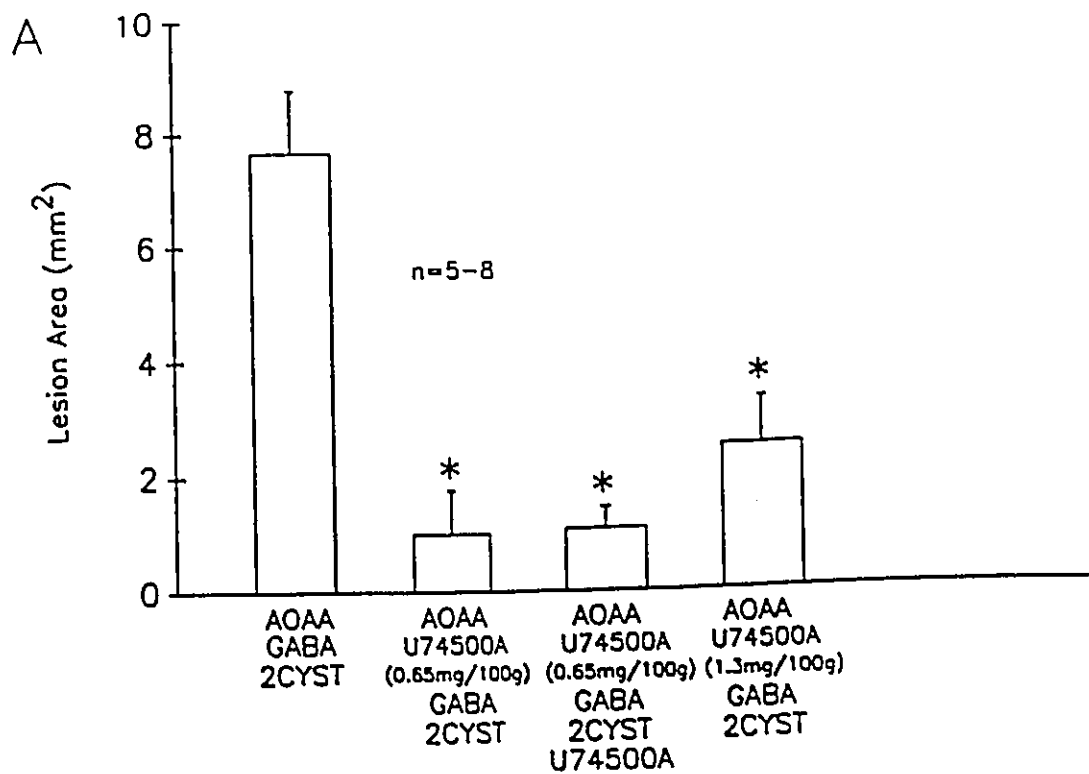
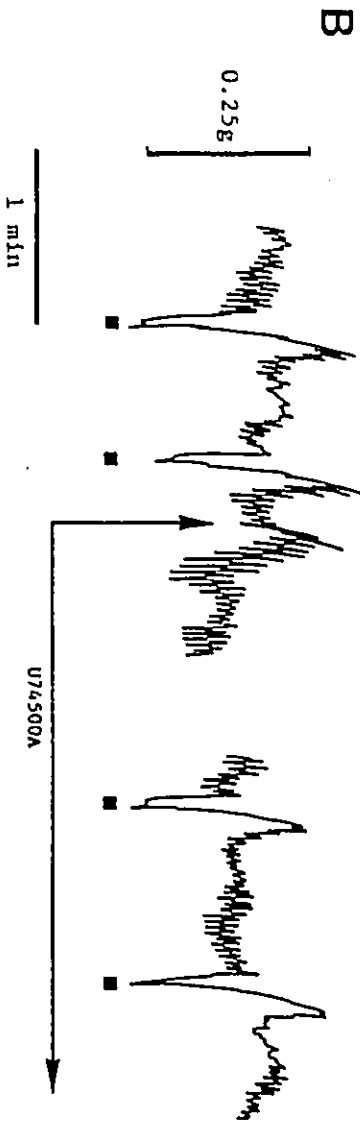
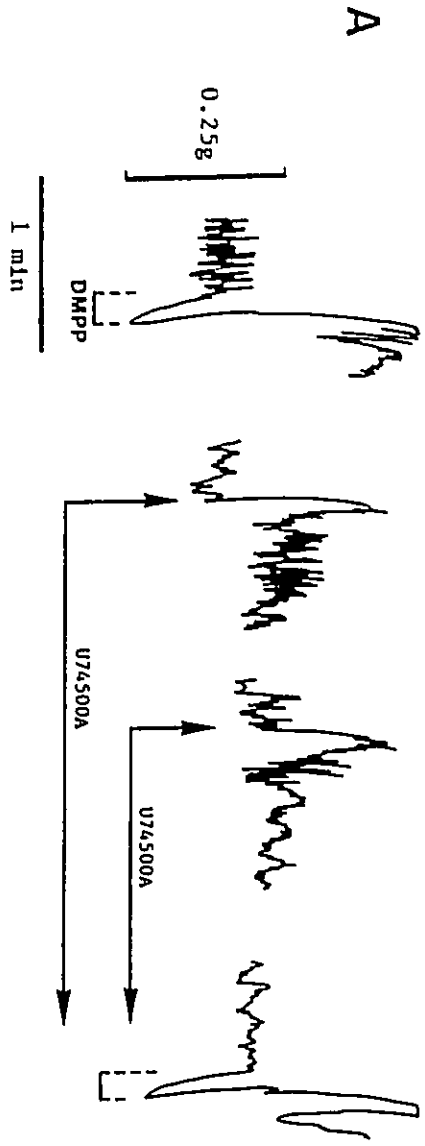


Figure 12. Original recordings of the effects of U74500A on evoked relaxations of the rat distal jejunum.

a) U74500A ($3.25 \times 10^{-5} \text{M}$) was administered (first arrow) and left in the bathing solution, and the tissue allowed to recover to resting level before challenge (second arrow) with an identical concentration. In the presence of U74500A (final concentration: $6.5 \times 10^{-5} \text{M}$), the relaxation response to DMPP (10^{-5}M), applied 7 minutes after the second challenge of U74500A, was not different from the control DMPP response.

b) Electrically-evoked relaxations (5Hz, 1msec) of the rat jejunum in the absence and presence of U74500A ($6.5 \times 10^{-5} \text{M}$, connected arrows). U74500A was in contact with the tissue for 12 minutes before re-applying electrical stimulation (■). The duration of contact for DMPP is shown by the horizontal bars. The vertical bars correspond to the deflection generated by a force of 0.25g. The time bar represents 1 minute. Washout artefacts are sometimes evident.



presence of U74500A. When U74500A ($3.25 \times 10^{-5} \text{M}$) was left in the bathing solution and the tissue allowed to recover to the resting tension, the contraction response of the jejunum to a second, identical concentration of U74500A was completely unaffected (Figure 12a). In addition, exposure of the duodenum ($n=3$) or the jejunum ($n=4$) to high concentrations of U74500A ($6.5 \times 10^{-5} \text{M}$), for up to 40 minutes, did not affect the ability of the intestine to relax following the administration of the nicotinic, cholinergic agonist DMPP (10^{-5}M , Figure 12a) or electrical stimulation (5Hz, 1msec) of the intrinsic, NANC inhibitory motor nerves (Figure 12b). Histamine-induced relaxations of the small intestine (not shown) were also unaffected by U74500A (10^{-3}M).

Contractions of the proximal duodenum and distal jejunum induced by the muscarinic agonist carbachol, were significantly reduced ($p < 0.05$, $n=4-10$) in the presence of U74500A ($6 \times 10^{-5} \text{M}$), Figures 13 and 14. Three different concentrations of carbachol were administered (EC_{30} : $2 \times 10^{-7} \text{M}$; EC_{50} : $4 \times 10^{-7} \text{M}$; EC_{70} : $6 \times 10^{-7} \text{M}$), and in the presence of U74500A, the magnitude of reduction of the carbachol-induced contractions was similar. Furthermore, U74500A ($6 \times 10^{-5} \text{M}$) reduced ($p < 0.05$, $n=9$) 5HT ($3 \times 10^{-8} \text{M}$)-induced contractions in these preparations (Figure 15).

2.3.7 Summary.

The results of this study show that the lazaroid U74500A has protective,

Figure 13. The effects of U74500A ($6 \times 10^{-5} \text{M}$) on carbachol-induced contractions a) $2 \times 10^{-7} \text{M}$, b) $4 \times 10^{-7} \text{M}$ and c) $6 \times 10^{-7} \text{M}$ of the rat proximal duodenum. Data is expressed as the mean \pm SEM from a minimum of 3 tissues. Asterisks (*) indicate the statistical significance for the various treatments.

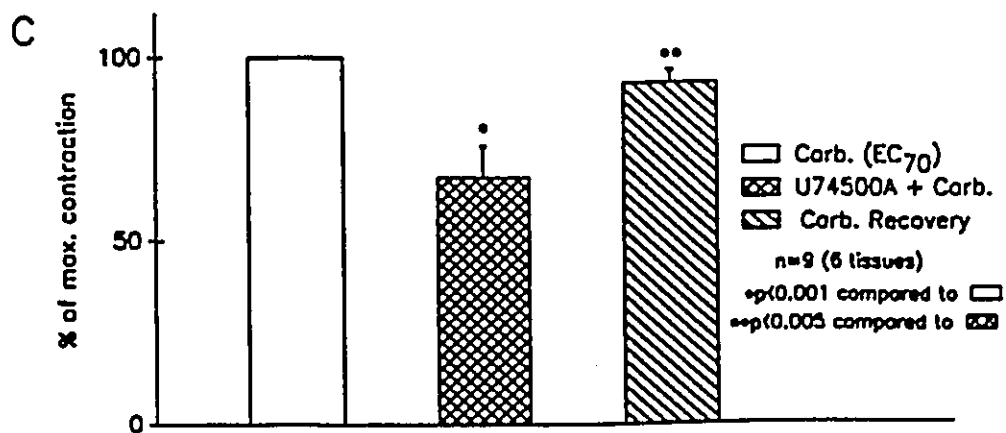
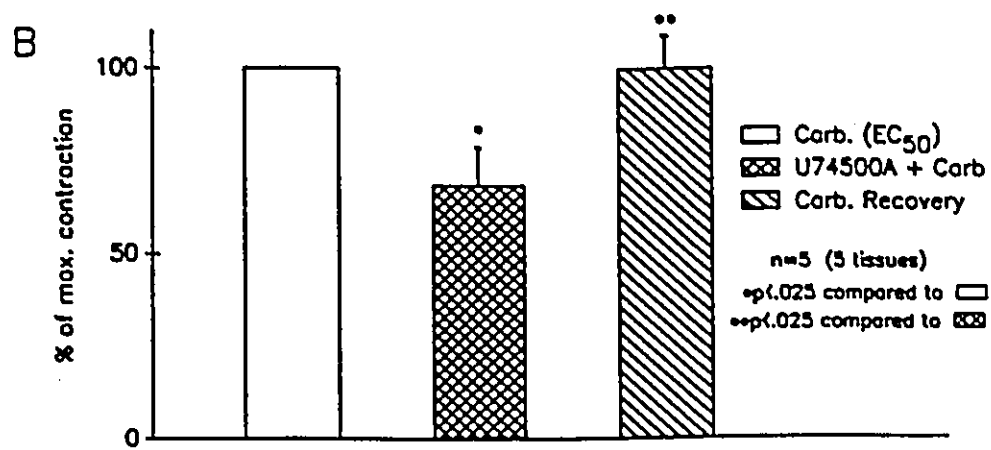
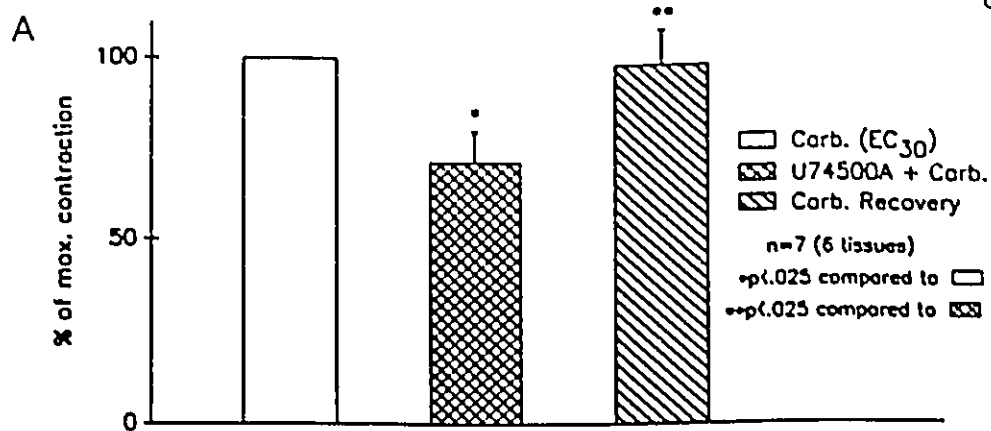


Figure 14. The effects of U74500A ($6 \times 10^{-5} \text{M}$) on carbachol-induced contractions a) $2 \times 10^{-7} \text{M}$, b) $4 \times 10^{-7} \text{M}$ and c) $6 \times 10^{-7} \text{M}$ of the rat distal jejunum. Data is expressed as the mean \pm SEM from a minimum of 3 tissues. Asterisks (*) indicate the statistical significance for the various treatments.

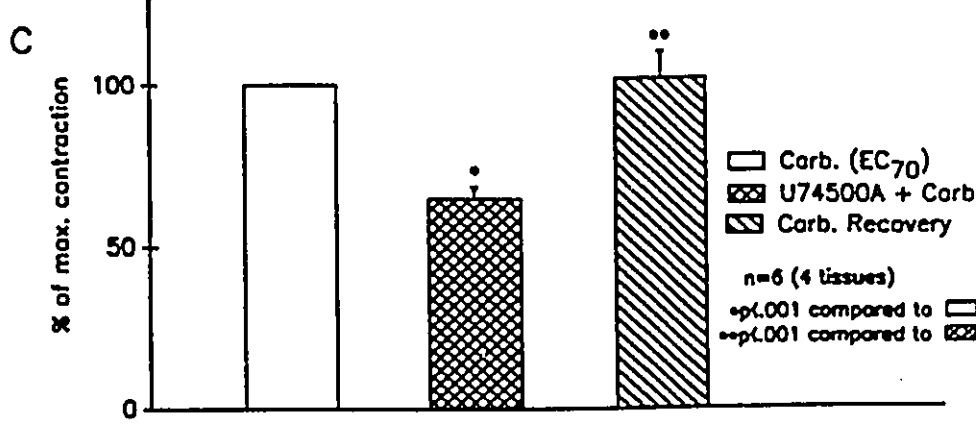
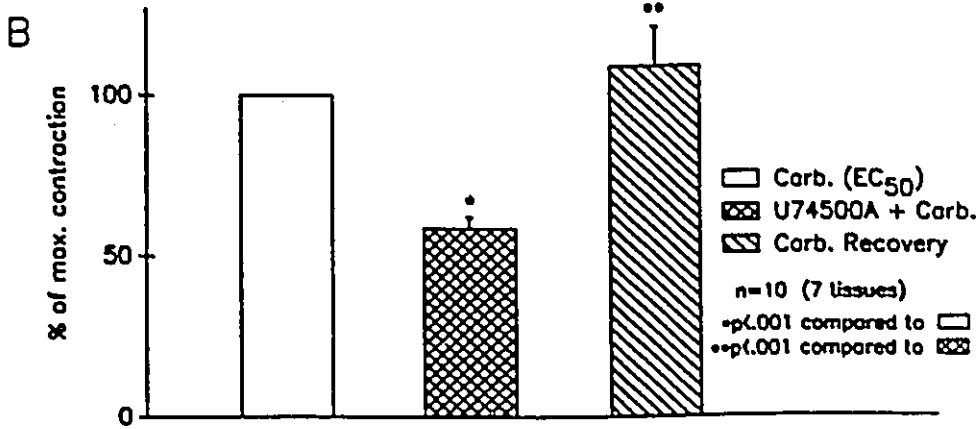
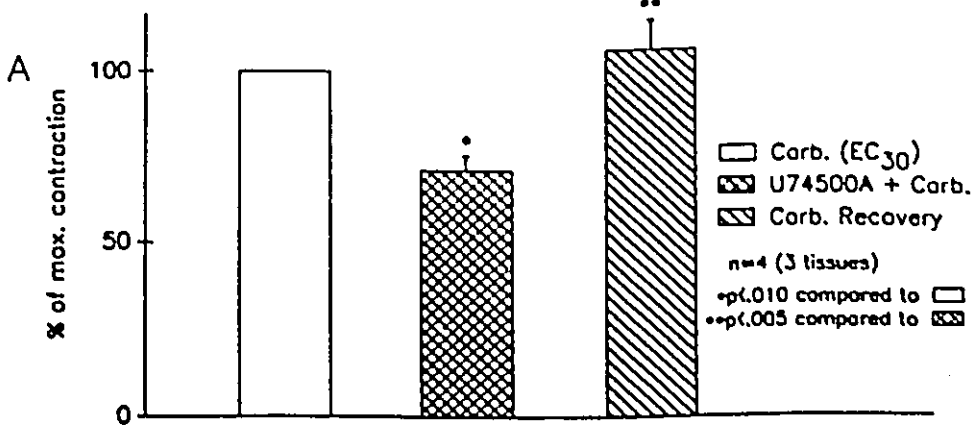
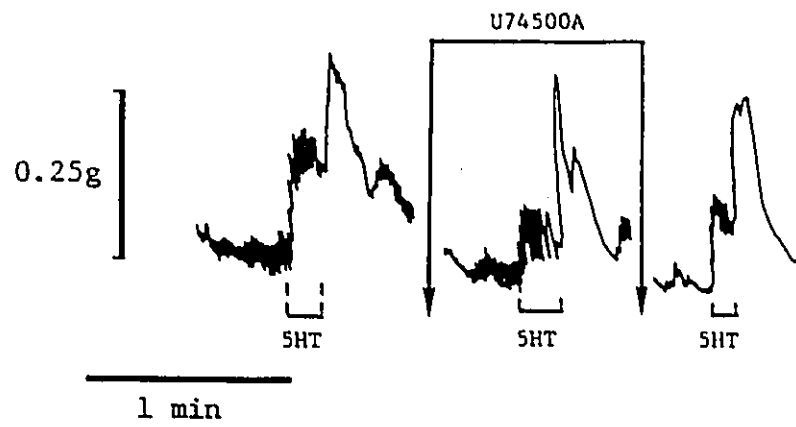


Figure 15. An original recording showing the effect of U74500A on 5HT-induced responses of the rat proximal duodenum. The contraction evoked by 5HT ($3 \times 10^{-9} \text{M}$) was reduced in the presence of U74500A ($6 \times 10^{-5} \text{M}$, connected arrows). The duration of contact for 5HT is indicated by the horizontal bars. The vertical bar corresponds to the deflection generated by a force of 0.25g. The time bar represents 1 minute. Washout artefacts are evident.



anti-ulcer actions against cysteamine-HCl and GABA-aggravated cysteamine-HCl induced duodenal ulceration. Moreover, U74500A appears to have more powerful, anti-ulcer actions than the H₂-receptor antagonist cimetidine. The mechanism(s) of this anti-ulcer action of U74500A is presently unknown. However, the results obtained from the in vitro organ bath experiments described above show that U74500A interacts and/or interferes with cholinergic muscarinic and serotonergic mechanisms.

Anti-muscarinic compounds have previously been shown to be effective in reducing gastric acid secretions, and these actions may partly explain the powerful, anti-duodenal ulcer effects of this lazaroid. The possibility also exists that U74500A exerts a more generalized action on gastrointestinal smooth muscle, since this compound reduced serotonin-induced contractions but had no effect on relaxations evoked by histamine or electrical stimulation. Moreover, U74500A may exert neurohumoral actions which could influence the ulceration process, since this drug effectively reversed the aggravating actions of GABA on the cysteamine-HCl induced ulceration.

These results suggest that these new lazaroid compounds may represent a potentially useful, anti-duodenal ulcer therapy. Furthermore, U74500A may exert its effects via mechanisms distinct from that of current drug therapies, such as cimetidine. On this basis, future studies should seek to determine whether the actions of U74500A and cimetidine are additive.

It has become increasingly apparent that more than one factor, associated

with duodenal ulceration, needs to be pharmacologically addressed for appropriate treatment of this disease. The present study provides evidence for two potentially new and effective anti-duodenal ulcer compounds, U74500A and baclofen.

Although baclofen has previously been observed to have contradictory effects on gastric acid secretion, it is highly unlikely that this drug has stimulatory actions since baclofen effectively reduced all measured parameters of cysteamine-HCl induced duodenal ulceration.

U74500A blocked carbachol-induced contractions of the small intestine, suggesting that this drug may have anti-muscarinic actions. Anti-muscarinic compounds (ie. telenzepine and pirenzepine) have been demonstrated to be effective in reducing gastric acid secretion (Kromer and Gönne, 1988). However, in these same studies, both cimetidine and the H⁺/K⁺-ATPase inhibitor omeprazole inhibited cysteamine-HCl induced acid secretion to the same degree as the anti-muscarinic agents. This data suggests that the observed anti-ulcer actions are not only due to anti-secretory effects.

In both cases, the possibility exists that the anti-duodenal ulcer effects observed with baclofen and U74500A may be mediated through actions on gastroduodenal motility. Although contradictory evidence pertaining to the actions of baclofen on motility has been obtained from in vivo and in vitro studies, it is clear that baclofen can influence gastrointestinal motor activity.

Baclofen has previously been shown to have a prejunctional, inhibitory mechanism of action, blocking enteric, excitatory cholinergic motor neurons (Ong

and Kerr, 1983; Giotti et al, 1983; Krantis and Harding, 1987). From the present in vitro studies, it would appear unlikely that U74500A exerts its actions on motility in a similar way.

The current in vitro study shows U74500A to exert actions on gastrointestinal motility, reducing 5-HT evoked contractions, but ineffective against histamine or electrically stimulated relaxations of the rat small intestine. In addition, U74500A may diminish contractile activity through a more generalized action on the gastrointestinal smooth muscle.

These studies demonstrate that a role for motility is becoming increasingly important in cysteamine-HCl induced duodenal ulceration. Moreover, motility disruptions have been shown to occur prior to any evidence of secretory, biochemical and functional changes, and actual lesion formation (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). Based on the current evidence, new therapeutic approaches may involve the use of these compounds to correct motor dysfunction in conjunction with the various anti-secretory agents.

CHAPTER 3

Secretory changes, including hypersecretion of gastric acid and impaired duodenal neutralization, are clearly important in the pathogenesis of duodenal ulceration. The ulcerogen cysteamine-HCl has been shown to increase gastric acid secretion and decrease duodenal HCO_3^- secretion, with lesion formation on the mucosal surface evident within six hours (McIntosh et al, 1984; Bridén et al, 1985; Takeuchi et al, 1987). However, the subcutaneous administration of cysteamine-HCl in rats has previously been observed to abolish gastric contractions, induce duodenal hypermotility, alter myoelectric activity and decrease slow wave frequency (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989).

It is known that these alterations in gastrointestinal motility precede the secretory changes and development of duodenal ulcers. The pharmacological actions of cysteamine-HCl have also been examined in in vitro organ bath preparations of the rat and guinea-pig small intestine. Cysteamine-HCl increased, in a concentration-dependent manner, the tone and contractile activity of the rat gastroduodenum and guinea-pig ileum (Bakich et al, 1984; Pihan et al, 1985). According to these studies, the actions of cysteamine-HCl were mediated through intrinsic cholinergic neurons and by direct actions at muscarinic receptors in the muscularis. However, more recently it has been shown in the rat gastrointestinal

tract that the cholinergic contractions induced by cysteamine-HCl are due primarily to myogenic rather than cholinergic, nerve-mediated mechanisms (Krantis, 1987; Krantis and Krause, 1989). Furthermore, these induced contractions were present in the stomach but not the intestine. The primary action of this ulcerogen in the rat small intestine was to evoke concentration-dependent relaxations through stimulation of the intrinsic, inhibitory, non-adrenergic non-cholinergic (NANC) motor neurons. According to Krantis and Krause (1989), previous studies failed to observe the relaxant actions of cysteamine-HCl due to the 'low tone' of the tissue preparations. In addition, cysteamine-HCl was also found to interfere with stimulation of these same inhibitory neurons by GABA and the nicotinic, ganglionic agonist DMPP, but not electrical stimulation. Furthermore, this ulcerogen reduced electrically evoked cholinergic nerve-mediated contractions, and blocked the actions of 5-HT at the muscularis. Neither carbachol nor histamine stimulation of the muscularis were affected by cysteamine-HCl, suggesting that the anti-cholinergic, anti-5-HT actions induced by this ulcerogen were unrelated to a disruption of excitation-contraction coupling per se.

Studies by Krantis (1987) and Krantis and Krause (1989) in the rat, imply that there may be up to three distinct neurogenic sites through which cysteamine-HCl exerts its effects. These include stimulatory sites on intrinsic NANC inhibitory neurons; inhibitory sites on intrinsic NANC inhibitory neurons and inhibitory sites on intrinsic cholinergic motor neurons. Thus, cysteamine-HCl, through interactions with enteric motor neurons could disrupt gastrointestinal motility, resulting in

alterations which may be important in the pathogenesis of experimental duodenal ulcer disease. In fact, such alterations of motor activity may be the primary precipitating condition for the subsequent development of duodenal lesions within the duodenal bulb. Therefore, the relationship between gastroduodenal motor activity and the development of duodenal ulceration in vivo needs to be investigated.

In vitro and in vivo recording techniques have previously been employed to examine gastroduodenal motility. In vitro techniques have been proven to be valuable in gastrointestinal motility studies, allowing cellular mechanisms and sites of actions to be examined. These types of studies overcome certain ethical and technical problems associated with in vivo experiments, but it has to be determined how well the in vitro situation correlates to the in vivo situation (Sanger and Bennett, 1982). In vitro recording techniques have the advantage that the system can be controlled, such that accuracy and reproducibility of the responses is possible (Sanger and Bennett, 1982). However, this method may not reflect motility patterns under normal, physiological conditions since the effects of hormones and other substances which reach the gastrointestinal tract through the blood are not present. Under in vitro conditions, the isolation of the tissue may result in damage to the preparation, and associated degenerative changes may affect the motility recording. In addition, the method used to sacrifice the animal may influence the responsiveness of the muscle, particularly in the case of euthanasia by anaesthetic overdose. Enteric smooth muscle and neural innervation are very susceptible to

opioid anaesthetics.

Many different in vivo techniques have been employed in recording gastrointestinal motility in mammals. One of the earliest approaches used intraluminal balloons. However, the balloon was found to both interact and stimulate the gut wall, resulting in a modification of the mechanical responsiveness of the gut (Corazziari, 1982). Therefore, this system was not suitable for recording intraluminal pressure changes, even when the system was connected to strain gauge pressure transducers. Furthermore, the luminal placement of the balloon catheter caused an obstruction to the gastrointestinal tract.

Manometric catheters have been used in in vivo studies to examine motility, with less interference of gut motor activity (Corazziari, 1982). Manometric pressure recordings have been obtained during the development of duodenal ulcer in normal and diseased patients (Monto et al, 1974; Borgstrom et al, 1978; Dooley et al, 1985). Unfortunately, these studies have given rise to conflicting data. The recording openings of the manometric catheters have a tendency to become occluded by mucosa, preventing the transmission of intraluminal pressures (Harris et al, 1966). Although this problem has been overcome by the continuous perfusion of the catheter with distilled water, the recording is dependent on the infusion rate, as well as the compliance and physical characteristics of the catheter system being employed (Corazziari, 1982). More recently, manometric studies have employed intraluminal transducers and greater accuracy has been found with these recordings (Hay et al, 1979). Unfortunately, intraluminal transducers are

very expensive and have a short lifetime compared to external transducers, making this method less practical to use (Corazziari, 1982).

In vivo motor function has also been examined using radiotelemetry capsules. These minute, pressure transducer containing capsules are swallowed and transmit radio signals at a frequency which is modulated by the variation in intraluminal pressures (Farrar et al, 1957; Farrar and Bernstein, 1958). The major disadvantage of this technique is with the mobility of the capsules. It is not possible to prolong the duration in which the capsule is in a particular region of the gut. This technique has most often been used in human studies.

More recently, extraluminal strain gauge force transducers have been developed for attachment to the serosal surface of the gastrointestinal wall. This in vivo approach is particularly useful to study motility patterns in unrestrained, conscious animals and has many advantages over the older, more traditional recording methods. These strain gauge force-displacement transducers are devices which change their electrical properties in response to deformation of their shape, and allow activity of either the circular or longitudinal muscle layers to be recorded, depending on the orientation of the gauge. Recordings can be obtained without interference of the intraluminal contents or stimulation of internal receptors (Bass and Wiley, 1972). Furthermore, the gauges are fixed permanently into position on the serosa, and therefore, allow multiple recordings from multiple sites in the same region of the gut.

The initial strain gauge force transducers were developed for use mainly in

studies involving larger animals such as dogs (Ludwick et al, 1968) and monkeys (Weisbrodt et al, 1971). In 1978, Pascaud et al developed a miniature extraluminal strain gauge force transducer for chronic recording of motility in the rodent. Pascaud et al (1978) found that in conscious, unrestrained rats, a good relationship existed between intraluminal pressure waves recorded by an intraluminal balloon system and the motor activity of the gut using the extraluminal strain gauge force-displacement transducers. In contrast to the balloon pressure system, the serosal strain gauges are relatively free of movement artifacts that plague balloon use, even during feeding and defecation (Pascaud et al, 1978). Their results proved both the reliability and sensitivity of the serosally mounted miniaturised strain gauges. However, these miniature strain gauge force transducers required considerable assembly for construction.

Many problems have been associated with the earlier in vivo recording techniques. In many situations, anaesthetized animals have been used which does not allow proper analysis of motility patterns under normal, physiological conditions. In addition, these older recording techniques do not detect gastrointestinal relaxations. This is important since motility is a complex but coordinated series of motor events which includes both smooth muscle contractions and relaxations.

Therefore, the aim of these studies was to develop an in vivo recording method to examine gastroduodenal motility in conscious, unrestrained rats. I **sought to test the hypothesis that intrinsic, neural pathways involved in**

propulsive motor activity of the gastroduodenum are disrupted during the development of duodenal ulceration.

In order to address these questions, I undertook to: 1) develop a reusable strain gauge recording device and a surgical procedure for the placement of these recording devices in the gastroduodenum; 2) develop a method for rapid analysis of data obtained from multiple recording sites; and 3) examine gastroduodenal motility in vivo, in conscious, unrestrained rats. The results of these studies are reported in three major sections (3.1, 3.2 and 3.3).

The first study (section 3.1) details features of the strain gauges, and describes the surgical and experimental protocols used in the in vivo motility recordings.

The second study (section 3.2) explains the approach used in the data acquisition and subsequent data analysis. In addition, the method for distinguishing contractile and relaxant motor activity has been described.

The final study (section 3.3) consists of 3 separate series of experiments. The first series of experiments (3.3.1) were designed to examine gastroduodenal motility under control conditions to determine the normal patterns of motor activity. The second series of experiments (3.3.2) investigated the actions of cysteamine-HCl on motor activity of the gastroduodenum. The third series of experiments (section 3.3.3) sought to determine the effect of systemically administered γ -aminobutyric acid (GABA) on the cysteamine-HCl induced effects on gastroduodenal motility.

3.1 Development of an *In Vivo* Motility Recording Technique and Computer Analysis.

The aim for this portion of the study was to develop a reliable and simple but inexpensive method of recording gastroduodenal motility in vivo in conscious, unrestrained rats. Based on the literature, miniature extraluminal strain gauges appeared to be the least invasive and most suitable approach for recording gastroduodenal motility in unanaesthetized rats.

3.1.1 Strain Gauges

Inexpensive, foil strain gauges were selected such that minimal construction was required for subsequent embedding to the gut wall. All in vivo gastroduodenal motility recordings were obtained using Showa foil strain gauges purchased from Durham Instruments (Pickering, Ontario). The specific characteristics of these strain gauges are shown in Table 3.

Following the selection of the type of strain gauge to be used, different types of wire (silver wire versus plastic insulated wire) were tested to determine which would be the most appropriate for connection to the strain gauge and most suitable to work with during the surgical procedure. Silver wire was found to be difficult to use due to its considerable flexibility. Plastic insulated wire (Alpha, 32 AWG) was a good alternative, being easier to manipulate during the surgical

Table 3. Characteristics of the Showa foil strain gauges used in the in vivo gastroduodenal motility recordings.

TYPE:	N11-FA-1-120-11
GAUGE LENGTH:	1 mm
RESISTANCE:	119.9 ohms
GAUGE FACTOR:	2.10±2%

placement of the gauges, and the coloured insulation allowed identification of each gauge within the gastroduodenum.

3.1.2 Construction of the Strain Gauges for *In Vivo* Gastroduodenal Motility Recordings.

Plastic insulated lead wires were connected to each strain gauge in the following way. The wire leads from the strain gauges were cut to an approximate length of 0.5-0.8cm and dipped into soldering paste (No. 1293, General Electric). A heated soldering iron was then applied to the leads for removal of the overlying thin plastic coating. Next, 32 AWG plastic insulated wire (approximate length 20cm) was soldered to each strain gauge lead wire. At this point in the assembly of the strain gauge unit, the resistance was approximately 120 ohms. The strain gauge units, consisting of the foil strain gauge, soldered joint and lead wires, were subsequently coated with Gagekote #8 liquid acrylic (Durham Instruments) for protection from body fluids. It was important to adequately coat the strain gauge unit and usually, a minimum of three thin coatings of the liquid acrylic was applied. A typical strain gauge with leads attached and coated with acrylic is shown in Figure 16. The coated strain gauge units, each colour coded for identification, were subsequently packaged for gas sterilization.

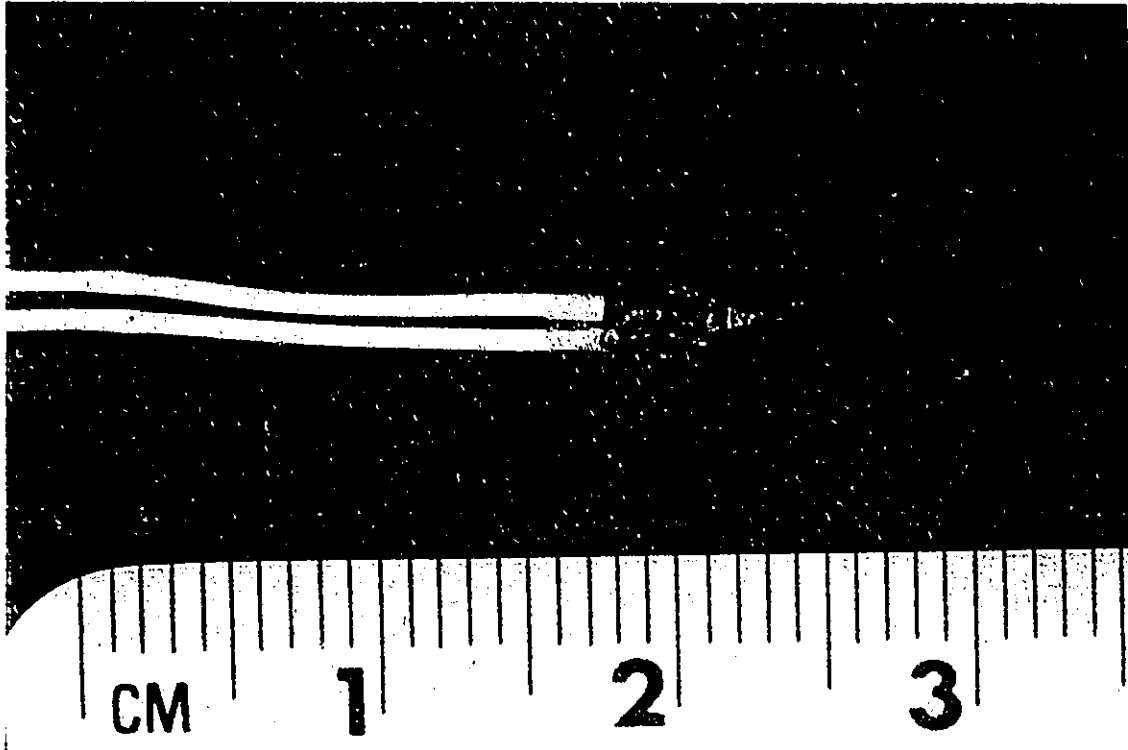


Figure 16. A photograph of a typical foil strain gauge (with attached leads and coated with acrylic) used in the in vivo gastroduodenal motility recordings of conscious, unrestrained rats.

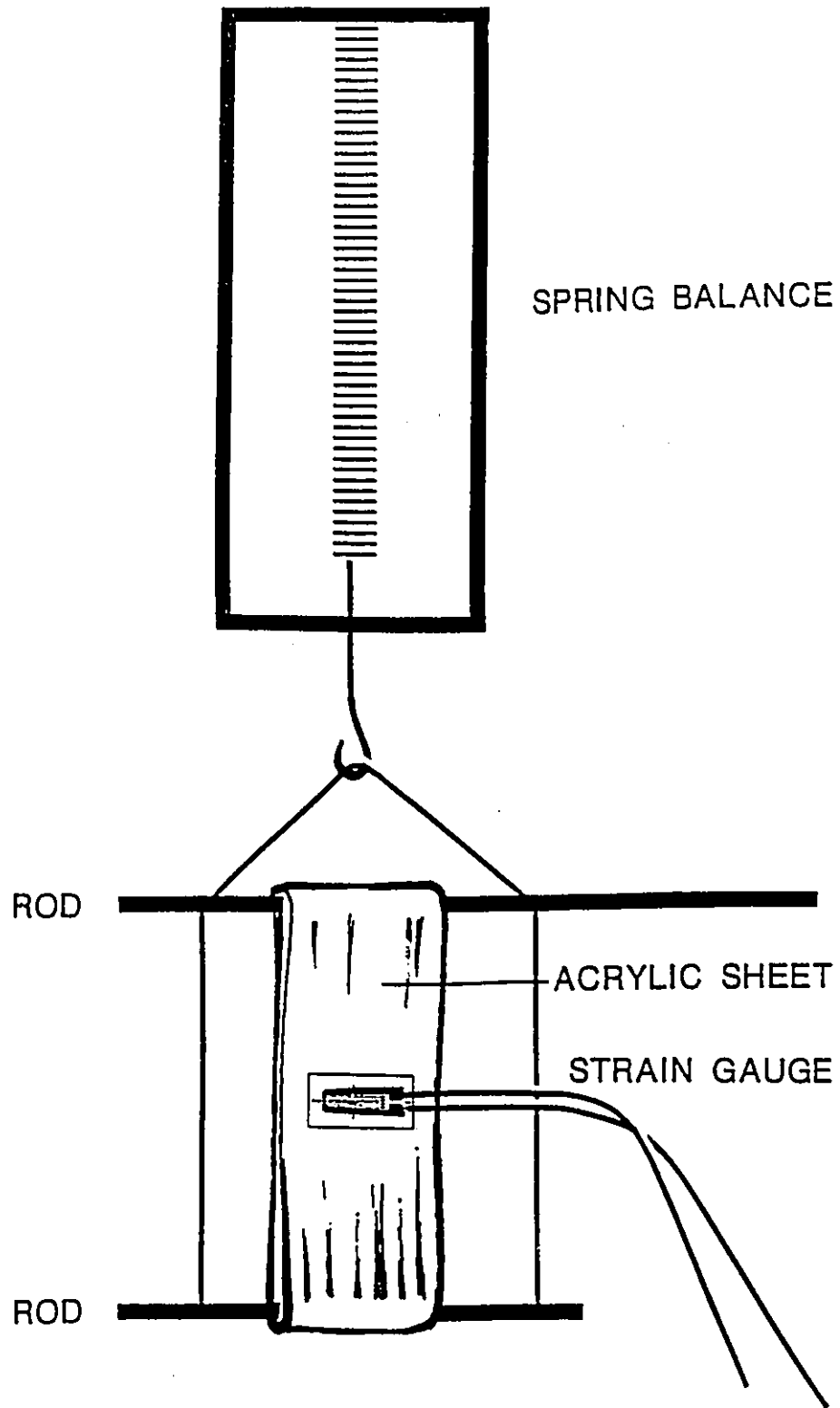
3.1.3 Calibration of the Constructed Strain Gauge Units.

Strain gauges were randomly chosen to undergo a calibration procedure to determine the linearity of resistance of the assembled strain gauge units. An artificial system was employed since it was not possible to physiologically mimic the gut wall. The system of calibration used in these studies represents a modification of the system used by Schuurkes et al (1977).

Three layers of 4.3 x 0.7cm acrylic sheets were glued together using clear marine silicone (General Electric). Two steel rods, each 1.0mm in diameter, were fastened and secured to the upper and lower portion of the acrylic sheets. Additional silicone was applied to the points where the acrylic sheeting and rods met for added strength and durability. Two holes had previously been drilled into each of the rods, 1.5cm and 2.5cm from the end. The upper rod was then fixed into a clamp. Silk (6-0) thread was fixed to each of the holes in the lower rod, and led through friction-free holes in the upper rod to a spring balance (Ohaus, 250g/9oz) which was located at a height of 9.8cm from the uppermost rod. A strain gauge unit was affixed, using marine silicone, to the surface of the acrylic sheets at the point of bending where tension (g) was applied. The silicone was allowed to dry for at least 24 hours. The complete set-up for calibration of the gauges is illustrated in Figure 17.

The strain gauge calibration assembly unit was aligned directly beneath the hook of the spring balance, with the exerted force directly read from the spring

Figure 17. Schematic illustration of the method used for the calibration of the Showa foil strain gauges. See section 3.1.3 for details.



balance. In the initial situation, the silk threads had a tension just greater than 0g so that they were not hanging slack. A brass knob was placed on the right end of the upper rod to allow rotation of the upper rod and thus, maintain alignment of the upper and lower rods during increasing gram tension. Force was exerted on the strain gauge by moving the spring balance upwards in increments of 10g.

The amplitude of deflection (mm) was plotted against the exerted force (g) and the line of regression was calculated. Strain gauges were routinely calibrated for 0-40g of tension. An average calibration curve for six strain gauges is shown in Figure 18. The coefficient of determination (R^2) was at least 0.9916. Moreover, each strain gauge was determined to be linear in response to the added gram tension within the range over which it was to be used.

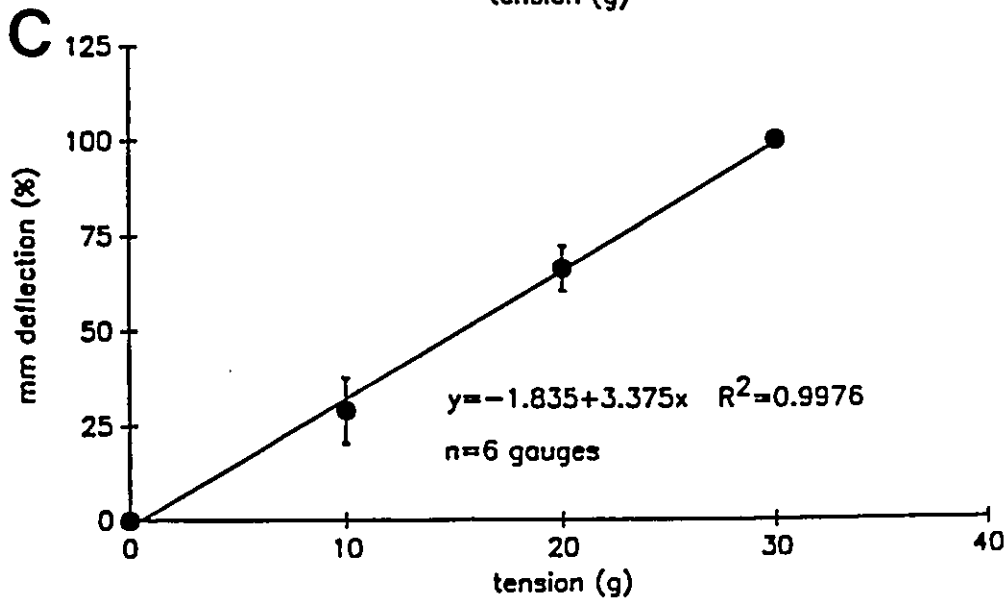
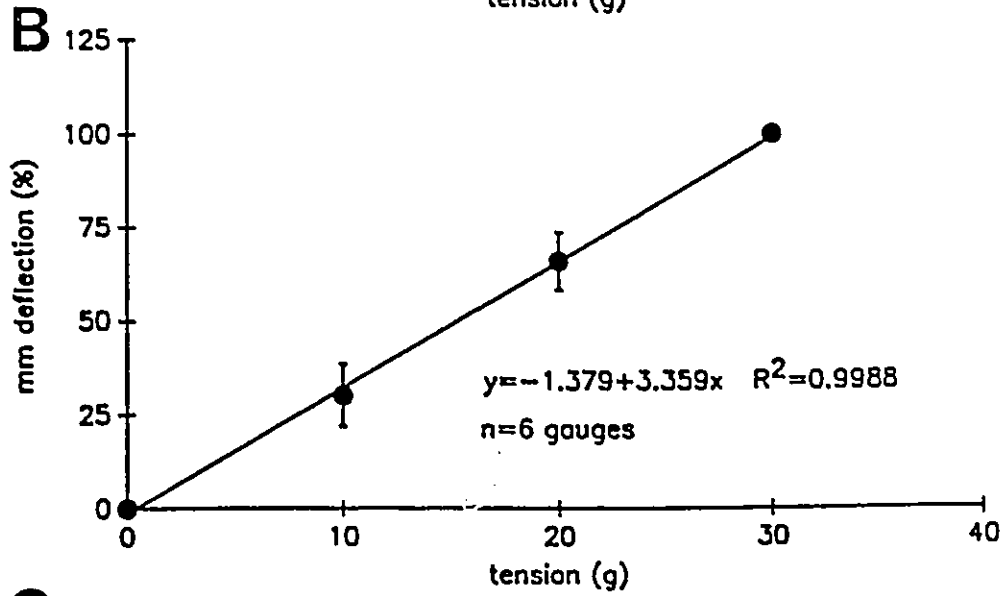
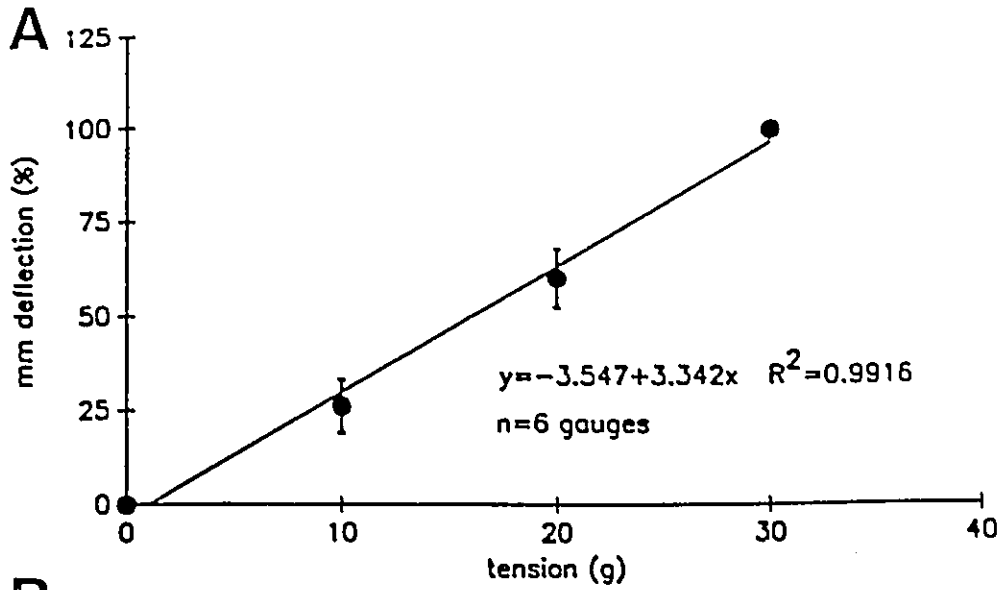
3.1.4 Surgical Placement of the Strain Gauges.

Male Sprague-Dawley rats (240-420g) were used in all experiments.

Training Schedule: Prior to the surgical placement of the strain gauges, all rats underwent a training period in metabolic cages which were similar to the actual experimental recording cage. The implementation of a training schedule allowed the rats to acclimate to the new surroundings and therefore, minimize behavioural disruptions during the recording period.

All rats were gradually accustomed to the recording conditions during a

Figure 18. Calibration curves obtained from six strain gauges from the a) antrum (S1); b) duodenum (D1) and c) duodenum (D2) recording sites. Data is presented as the mean \pm SEM. Each transducer was determined to be linear.



minimum five day consecutive training period. Rats had free access to both food and water during this time.

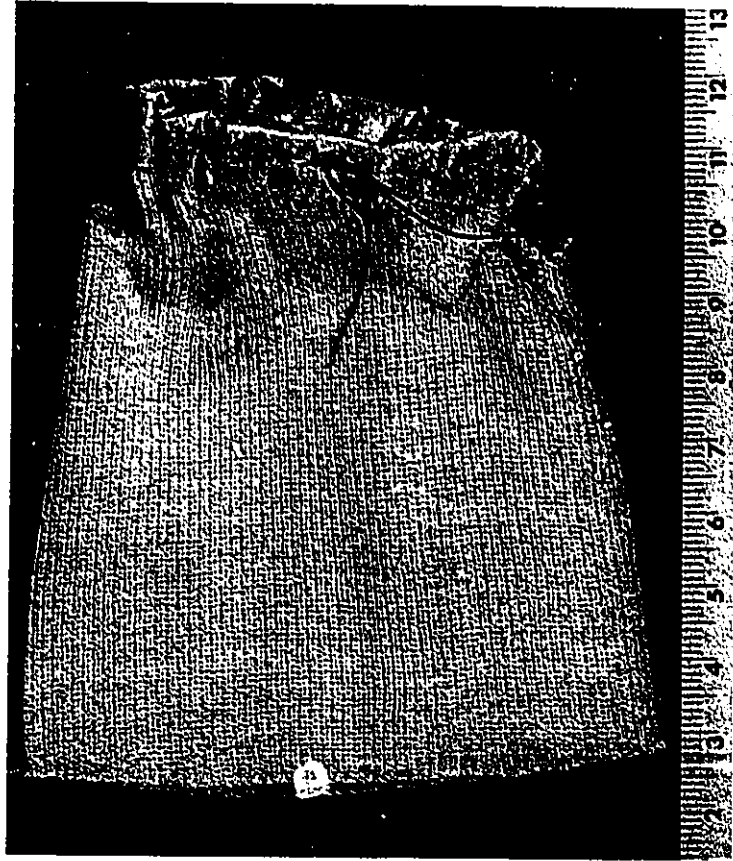
During each training session, the rats were dressed in both a stockinette jacket (Figure 19a) and an overlying custom (Alice King, California) jacket (Figure 19b). The jackets functioned to hold the exteriorized leads from the strain gauges in position on the dorsal region of the neck, and prevented the rats from chewing the leads following surgery. Therefore, the rats became accustomed to wearing the jackets prior to the surgical procedure.

On the first day of training, rats were dressed in both jackets and placed in a cage containing sawdust chips for two hours. The rat was periodically observed to ensure that there was no discomfort to the animal. After two hours of training, the jackets were removed and the rat was returned to a sawdust cage. From day 2 to day 5, rats were trained in metabolic cages, similar to the recording cage, for up to a maximum of eight hours.

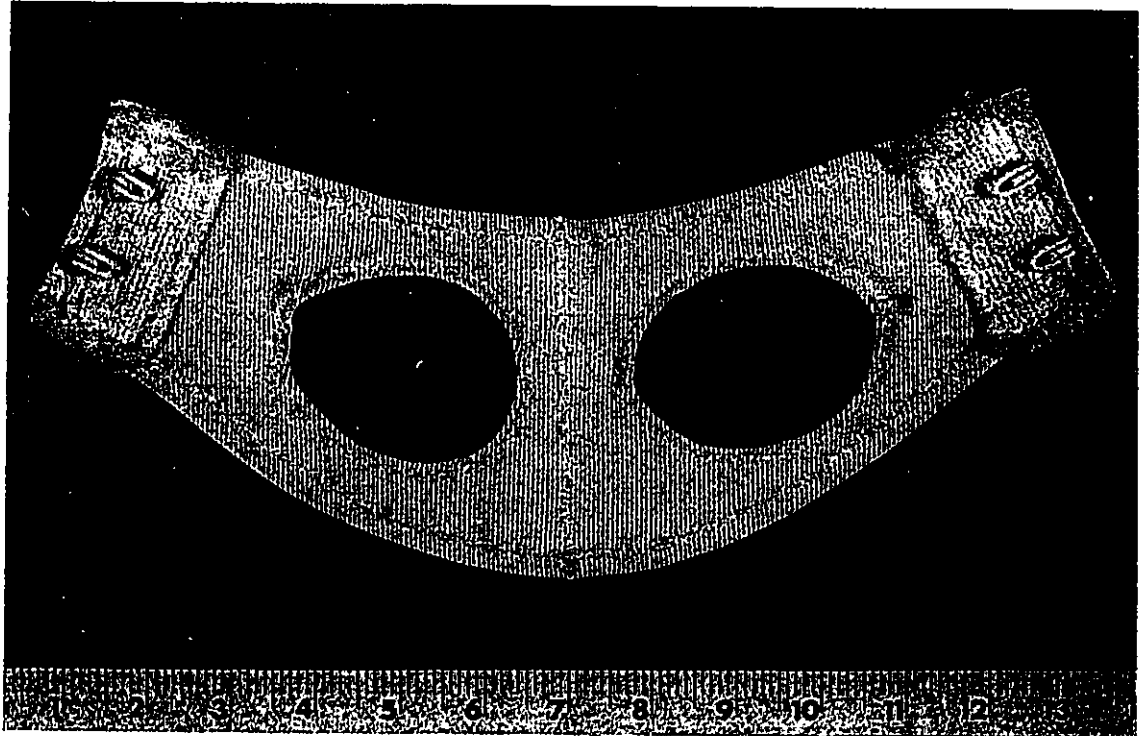
Rats were considered prepared for surgical placement of the strain gauges and subsequent recording of gastroduodenal motility after five days of training. Rats were removed from the study if they were not able to cope with the training protocol and/or showed signs of stress, such as mucus secretions around the eyes and/or nose. Rats would also be removed in the very early stages of the training routine if they appeared to be experiencing discomfort.

Figure 19. Photographs of the a) stockinette jacket and b) custom jacket (Alice King, California) worn by the rats during the training sessions.

A



B



Anaesthesia: Anaesthesia was administered to the rat with the aid of a veterinarian or a trained animal care technician. The rat was placed into a rodent anaesthetic box (21.5 x 15.0 x 13.5cm) which was attached to a small portable animal anaesthetic machine containing a proper scavenging Bain Circuit. The rat was administered a mixture of halothane (Fluothane, Ayerst) and oxygen (1.0-1.5%). Halothane was initially administered at 1% and then gradually increased to 3.5-4.0%.

Following induction of halothane anaesthesia, the animal was removed from the anaesthetic box and anaesthesia was maintained by using a small cat anaesthetic mask (outer diameter: 4.5cm; inner diameter: 2.5cm). Halothane was maintained between 1.5-2.5% and oxygen between 1.0-1.5%. The exact level of each gas depended on the animal care technician and the degree of stimulation to the rat.

Surgery: The abdomen of the rat was shaved (size 40 clipper blade, Oster) from the sternum to the pubis. The dorsal region of the neck was also shaved. A vacuum (Black and Decker) was used to remove any loose hair from the shaved regions.

The shaved areas were washed with an antibacterial skin cleanser, Hibitane (chlorhexidine gluconate 4% w/v), and rinsed with tap water. A topical germicide (1% free iodine) Betadine solution (10% povidone-iodine) was subsequently applied to the shaved regions. The rat was then placed into a sterile stockinette

to prevent contamination and possible infection during surgery.

Following preparation of the eventual skin incision site, the rat was transferred to an operating table and placed on a huck towel covered, solid aluminum ventilation table (30.0 x 15.4 x 3.8cm). This table was connected to a scavenging system to vent out any escaping halothane gas. A pump driven, hot water circulating heating pad was placed under the ventilation table to prevent severe hypothermia of the rat during the surgical procedure.

According to the University of Ottawa Animal Care guidelines, a veterinarian or animal care technician was always present to monitor the depth of anaesthesia throughout the surgical procedure, by periodic tail and/or toe pinches, level of respiration and responsiveness of the animal to surgical stimulation (ie. skin incision).

Stainless steel wound clips were used to secure the stockinette to the sterile huck towels, preventing movement of the stockinette and aiding in the sterility of the procedure. The stockinette was then cut open, approximately 6-7cm in length, to expose the shaved portion of the abdomen.

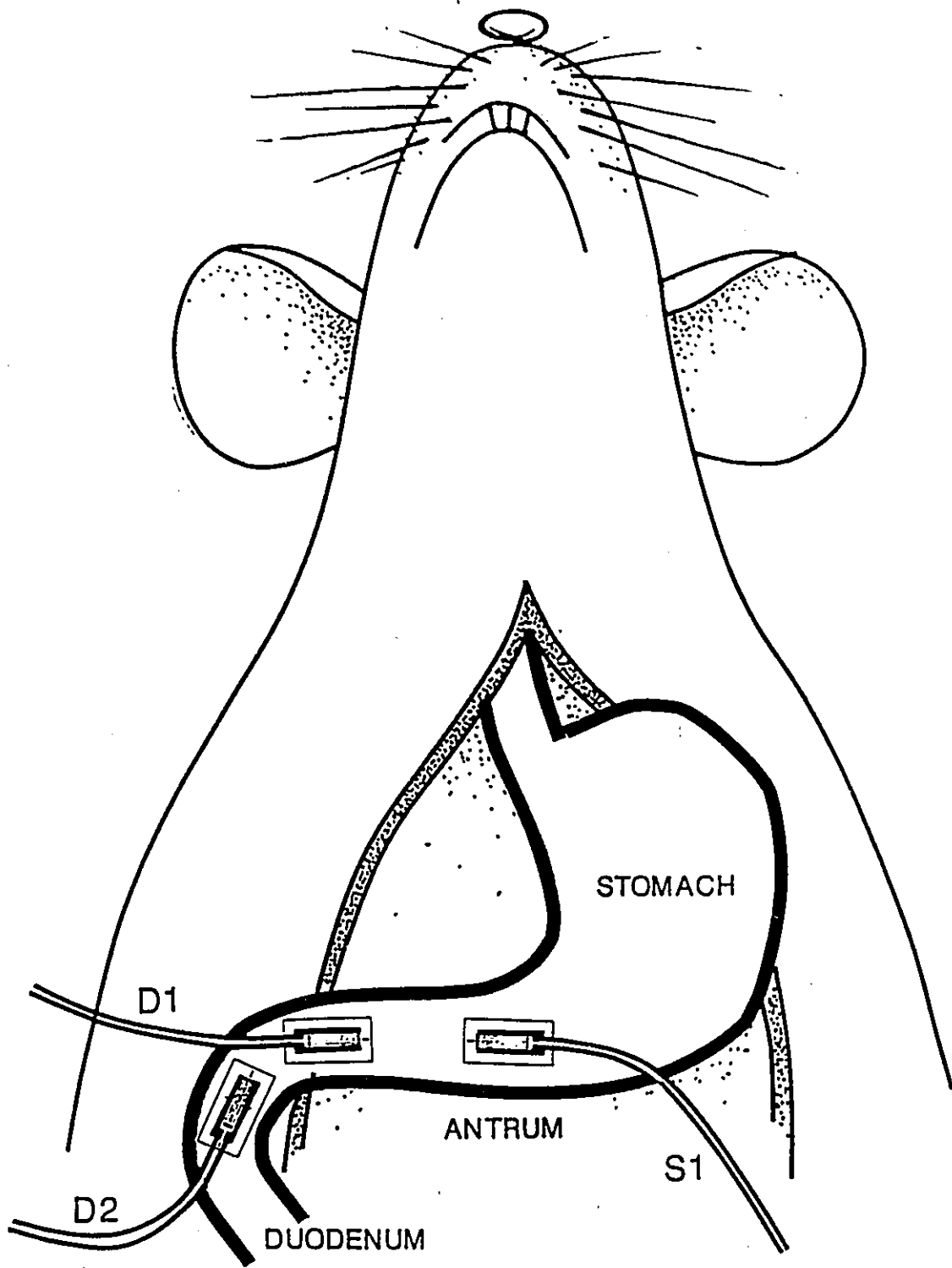
The initial skin incision (15 Rib-Back carbon steel surgical blade and number 3 scalpel handle) consisted of a middle laparotomy, approximately 4cm in length, to expose the peritoneal muscle layer. The peritoneum was then cut along the linea alba, allowing access to the stomach and proximal duodenum. The skin layer was separated from the muscle layer, using small curved forceps (Kelly); from the abdominal cavity to the dorsal region of the neck to allow for

subcutaneous tunnelling of the strain gauge leads. A blunt stab wound was made through the left and right abdominal muscles for the eventual passing of the strain gauge leads from the peritoneal cavity to the neck region.

For greater ease of placement of the strain gauges on the serosal surface of the gastroduodenum, the stomach and proximal duodenum were gently manipulated, pulled from the abdominal cavity and placed on saline-soaked sponges (5 x 5cm, Johnson and Johnson). After swabbing a small region of the antral surface of the stomach, the first strain gauge was positioned on the antrum, approximately 2cm proximal to the pylorus, using Vet-Bond glue (Tissue Adhesive, No. 1469, 3M Company). The Vet-Bond glue bonded the strain gauge unit to the serosal surface almost instantaneously. The serosal surface of the proximal duodenum was then carefully dried, and two strain gauges were positioned approximately 2cm and 4cm distal to the pylorus (Figure 20). The gauges were allowed to dry between 1.5-2.5 minutes and then, the gastroduodenum was repositioned in the abdominal cavity. The antral strain gauge leads were passed through the left abdominal muscles whereas the duodenal strain gauge leads were passed together through the right abdominal muscle, with slack wire remaining within the abdominal cavity.

Next, a small, longitudinal median incision (approximately 0.5cm) was made on the dorsal region of the neck so that the antral and duodenal strain gauge leads could be subcutaneously tunnelled from the abdominal cavity to the neck, and subsequently exteriorized.

Figure 20. Schematic illustration of the placement of the strain gauges on the serosal surface of the gastroduodenum for in vivo recording of motility. S1 represents the antral strain gauge, positioned 2cm proximal to the pylorus. D1 and D2 represent the strain gauges positioned on the proximal duodenum, 2cm and 4cm distal to the pylorus respectively.



The abdominal muscle was closed with synthetic, absorbable, sterile sutures (3-0 polyglactin 910-coated Vicryl, Ethicon Limited). The abdominal and neck skin incisions were closed using 9mm stainless steel wound clips (Autoclips, Clay Adams).

The exteriorized lead wires were wiped with 70% isopropyl alcohol and placed in a gauze pack which was secured to the rat. The rat was then dressed in a clean stockinette jacket, to ensure that the leads remained in the gauze pack overnight.

Recovery: Following completion of the surgery, the rat was maintained on pure oxygen until the animal showed signs of movement. Analgesia was not administered until the rat showed signs of recovery from the anaesthetic. This action prevented any accidental overdose to the rat. However, upon recovery from the anaesthesia, the rat was administered either Temgesic (0.1-0.2mg/kg sc, Buprenorphin-Hydrochloride; Reckitt and Colman) or Demerol (Meperidine-Hydrochloride; Winthrop). Demerol was given at a dose of 0.1ml/100g (1mg/1ml solution) subcutaneously or intramuscularly. Due to difficulty in obtaining Temgesic, maintenance of analgesia was switched to Demerol after the initial experiments. All rats received a 3cc dose of lactated Ringers solution (sc) either before or immediately after the surgical procedure. The rat was then transferred to a pre-warmed (37°C) infant incubator for approximately 4-5 hours before being returned to a sawdust cage. Rats were allowed a 24 hour period of recovery.

3.1.5 Experimental Recording.

Rats were active, alert, interested in the surroundings and demonstrated typical grooming behaviour 24 hours following the surgery. Furthermore, there was no evidence to indicate problems of infection at the two incision sites or within the abdominal cavity. On the basis of these observations, it was determined that the rats had made a complete recovery from the surgical procedure. The stockinette jacket and the gauze pack, containing the exteriorized leads, were removed from the animal. The resistance of all strain gauges was measured, with normally functioning gauges having a resistance between 120-122 ohms.

The exteriorized lead wires from the neck incision were connected to interface boxes which decreased the voltage from the Grass Polygraph (Model 7D). Segments of tygon tubing (ID: 1/8 inch; OD: 3/16 inch; wall: 1/32 inch) were placed over the exteriorized antral and duodenal strain gauge leads. The tubing functioned in a protective manner, holding the exteriorized leads together as a single unit, and deterring the rat from chewing through the leads. The rat was then placed into the recording cage.

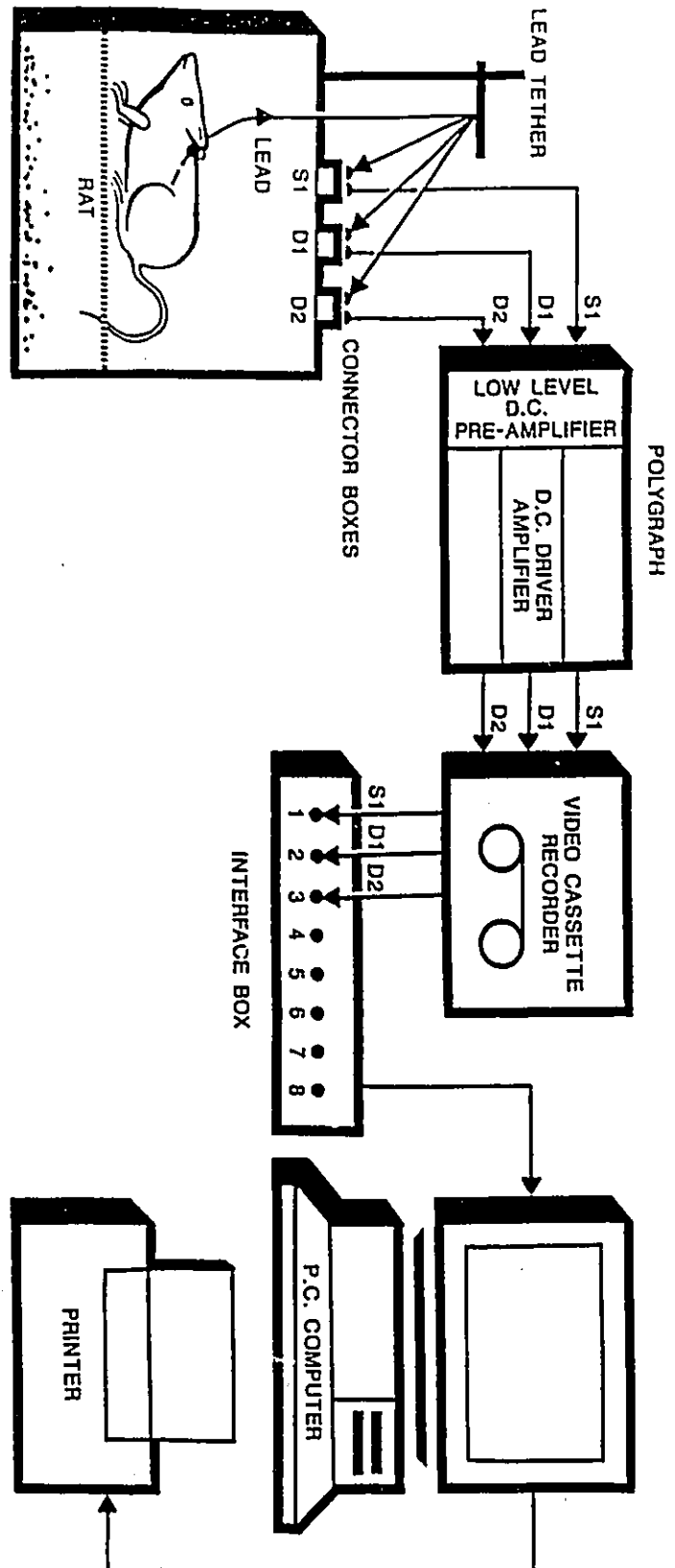
The recording cage consisted of a wire-bottom metabolic cage (25 x 20 x 17cm) suspended in a darkened metal frame. The frame of the cage was darkened with black construction board which reduced the rat's curiosity of its surroundings. The darkness of the cage also appeared to calm the animal. The front panel of the cage frame contained a clear plastic window (18 x 6cm) at the

level of the suspended metabolic cage, allowing the behaviour of the rat to be observed. It was important to be able to easily view the rat throughout the experiment so that artifacts in the recording due to movement of the animal could be appropriately noted. A sawdust containing reservoir was fitted into the bottom of the recording unit frame to catch the animal's excrements and prevent coprophagy. A metal rod (28cm height) was mounted onto the top of the cage frame which allowed the tension on the exteriorized strain gauge leads to be appropriately adjusted to ensure enough slack without restricting the movement of the animal. A slot on the top of the recording unit allowed the exteriorized strain gauge leads to be connected outside of the cage.

Gastroduodenal motility was also simultaneously recorded on video tape by a Vetter modified Panasonic Omnivision videocassette recorder (VHS PV-4760) for subsequent computer analysis (see section 3.2). Each experiment was recorded using new tapes to maximize the quality of recording and to eliminate the possibility of over-taping a previous experiment. The arrangement of the various components is illustrated in Figure 21.

During the recording sessions, the rat had no access to food or water, but had free movement within the recording cage. All motility recording sessions were performed in a quiet room. In addition, every recording session consisted of a three hour control recording period followed by drug treatments.

Figure 21. Schematic illustration of the system used for measuring gastroduodenal motility in the conscious, unrestrained rat.



3.1.6 Strain Gauges and Animals.

In the majority of experiments, all strain gauges were patent for the course of the experiment. When gauge failure did occur, it was very abrupt, and was usually due to the breakage of the fragile leads of the strain gauge itself. During the recording sessions, some baseline drift was evident, but this drift was usually due to some type of movement (grooming, stretching) of the animal within the recording cage. It was also determined that the orientation of the strain gauges in the circular muscle or longitudinal muscle direction did not result in different patterns of motility being observed. This was not unexpected since motility involves simultaneous responses of both smooth muscle layers.

Following sacrifice of the animal, the strain gauges were observed for the presence of adhesions. Adhesions were rarely observed, and could usually be detected during the recording session by an inability to properly balance the strain gauge.

3.1.7 Recovery of the Strain Gauge Units.

Following the completion of the motility recording session, the rat was overdosed with pentobarbital sodium (Somnotol, 65mg/kg ip). The abdomen was then opened, and the position of the strain gauge units checked. The gauges were carefully removed from the gastroduodenum and placed in a diluted ethanol

solution for 24 hours. Following the ethanol soaking, the strain gauges were clamped onto a microstand and allowed to air dry. Using a dissecting microscope (Zeiss), the liquid acrylic coating and glue layers were carefully removed from the units. Because of the fragility of the strain gauge leads, the acrylic coating was not removed from these wires. The gauges were then rinsed in alcohol, and allowed to air dry prior to the further application of the liquid acrylic coating. Each strain gauge could be re-used approximately three times before the build-up of acrylic on the fragile leads of the strain gauges became excessive.

3.2 Computer Analysis of Gastroduodenal Motility Recordings.

Quantitative analysis of enteric motor activity is a major problem in the study of gastrointestinal motility (Schemann et al, 1985; Schemann and Ehrlein, 1986). In particular, the large amounts of data collected makes visual/manual analysis of motility recordings a tedious, time-consuming process (Schemann et al, 1986; De Ridder et al, 1989). Unfortunately, there has been no simple method developed which allows for the immediate quantification of gastrointestinal motility recordings (Schemann et al, 1985; Hachet et al, 1986; De Ridder et al, 1989).

Previous studies using computer analysis to investigate gastrointestinal motility provided only limited analysis (Ehrlein and Hiesinger, 1982; Schemann et al, 1985; De Ridder et al, 1989). Computer analysis of motor activity has been for the most part, limited to single, quantitative measures such as the force or

frequency of contraction (Schemann et al, 1985). However, this type of analysis is not sufficient for the quantification of gastrointestinal motility patterns (Schemann et al, 1986). Therefore, the aim of this portion of the study was to develop a flexible, semi-automated computerized system for the rigorous analysis of gastrointestinal motility recordings obtained from conscious, unrestrained rats. **More specifically, I sought to develop software which would allow greater indepth analysis of motility patterns recorded from closely spaced, serosally positioned gastroduodenal strain gauges. In addition, this software should be 'menu-driven' for easy use by researchers.**

3.2.1a Data Acquisition

In order to quantify gastrointestinal motor activity, software had to be developed which allowed the acquisition of the recorded data (from the videocassette recorder) to an IBM-clone personal computer. Digitization of the data was attained by processing through an analog-to-digital (A/D) conversion board (DT-2808; Data Translation). The DT-2808 acquisition board had 10 bit resolution, such that if a 0 to 5 voltage difference was applied, a voltage difference as small as 4.88 millivolts could be detected.

The data acquisition program was written using ASYST software (see Appendix 1) and employed background/foreground multi-tasking. This operation allowed certain data acquisition and control tasks to run in the background at the

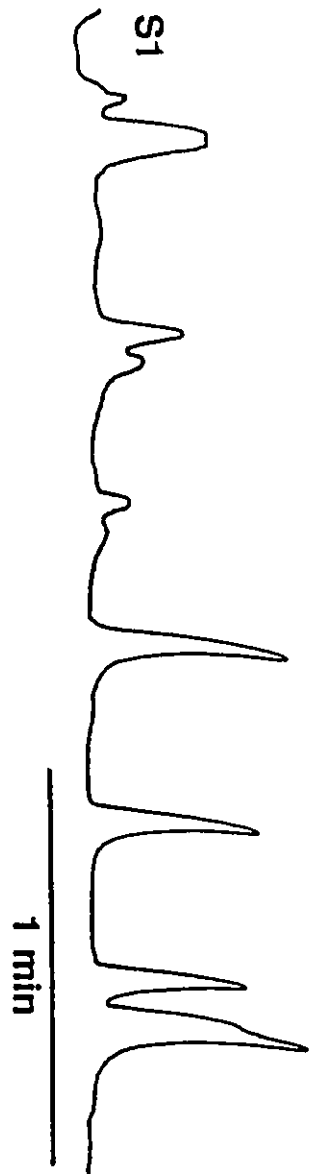
same time that it executed any other ASYST predefined subroutine in the foreground. Therefore, two or more procedures could be operating simultaneously.

In the data acquisition program, data was acquired from the videocassette recorder at a rate of 5 data points/second/channel from three data channels. The three data channels corresponded to the antral and two duodenal recording sites. The acquisition of data occurred in the background via two cycling buffers while plotting of these buffers occurred in the foreground at a rate of 50 data points/data channel every 10 seconds. Data from all recording sites was acquired and plotted simultaneously. The computer screen was divided into three portions (vuports) to allow the acquisition and plotting of data from the antral and duodenal regions to be visually observed. However, the number of vuports displayed was determined by the user. Within the program, the user inputted the rat identification number, VHS tape number, experiment number and treatment protocol at the appropriate command lines. Data obtained from the experiments was stored in data files within the computer in 160 minute epochs. All created data files were subsequently stored in files on 60 megabyte back-up tapes (3M Company).

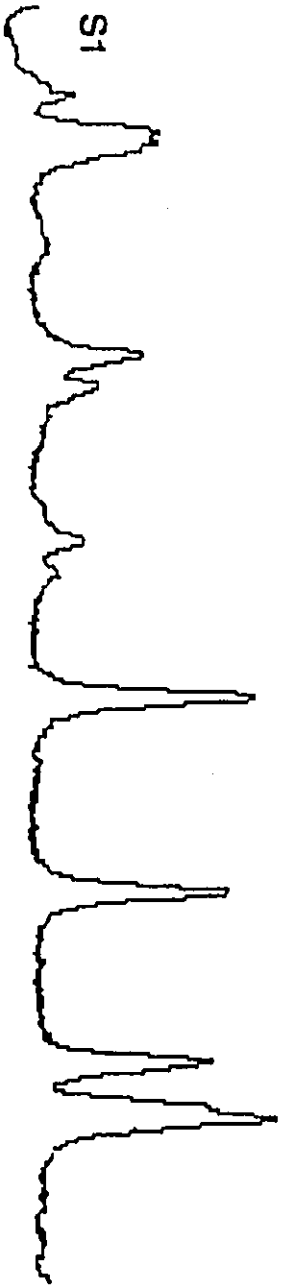
Figure 22 illustrates a typical real-time recording from the rat antrum compared to the computer-generated version following processing of the data through the acquisition program. The contractile events displayed by the computer were virtually identical to the real-time recording and demonstrated that the chosen rate of acquisition of the recorded data was appropriate for rat gastrointestinal motility. Therefore, there was excellent reproducibility of the motility recordings by

Figure 22. Comparison of a typical, real-time recording taken from the rat antrum to the computer-generated version of the same data. Good reproducibility exists between the two traces.

REAL TIME:



COMPUTER-GENERATED:



the computer.

3.2.1b Identification of Contractions and Relaxations.

The software developed for the quantitative analysis of motility recordings was done with the guidance of Dr. F. Johnson, Director of the Institute of Medical Engineering at the University of Ottawa. Programs were subsequently written in C language as it was determined that this language allowed greater programming flexibility than the ASYST software.

Previous studies examining gastrointestinal motility have only been able to record and measure contractile activity. However, a major advantage of our recording technique (see Section 3.1) is that both contractile and relaxant motor activity of the gastroduodenum can be measured.

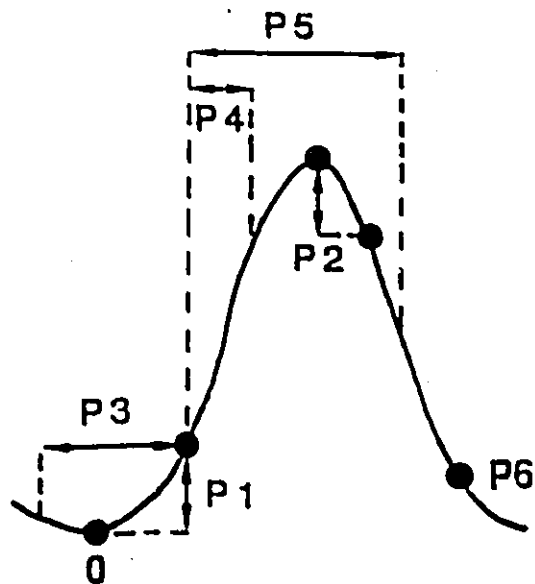
In order to perform quantitative analysis on the acquired data, contractile motor activity had to be distinguished from smooth muscle relaxations. In 1986, Schemann et al developed a method for recognition of contractions in recordings obtained from dogs. In this study, the method of Schemann et al (1986) was modified, so that both contractions and relaxations could be identified in the rat model (See Appendix 2 for computer program).

Contractions and relaxations were identified on the basis of threshold and timing criteria. Six different parameters were used to identify a contraction (P_1 - P_6) or relaxation (N_1 - N_6). A schematic representation of the principles used in the

detection of a typical contraction is shown in Figure 23. Although the method of detection has been described for a contraction, the approach is identical for recognition of relaxations. An average baseline (0) was established in all gastroduodenal recordings. The first parameter (P_1) represented a threshold for recognition of a contraction minimum. Thus, if the voltage value was below the P_1 threshold, the recorded response was not considered to be a true contraction. The parameter P_2 represented the threshold value for recognition of a contraction maximum. If the voltage value was greater than the P_2 threshold, the event was not a true contraction. P_3 was a time-window, in seconds, for the recognition of a contraction minimum. For a contraction to be considered a true event, the contraction must have reached the P_1 threshold within a designated length of time. The slope minimum was represented by P_4 , and this parameter was another time-window which limited the contraction rise time. P_5 was also a time-window which represented the slope maximum. The final parameter, P_6 , indicated the end of the response and the return to baseline levels.

To summarize, detection of a contraction minimum occurred when increasing voltage values exceeded the given P_1 threshold value within the P_3 time-window. Confirmation of a contraction maximum occurred when the decreasing voltage values exceeded the P_2 threshold value after the end of the P_4 time-window but prior to the end of the P_5 time-window. The defined threshold and time values of the parameters (P_1 - P_6) used in these studies resulted in the elimination of artifacts such that only genuine contractions and relaxations (using

Figure 23. Schematic representation of a typical contraction illustrating the threshold and time parameters (P_1 - P_6) used in the detection of contractile motor activity. See section 3.2.1b for details.



0 - BASELINE

P1 - THRESHOLD

P2 - THRESHOLD FOR PEAK MAXIMUM

P3 - TIME (S) FOR PEAK MINIMUM

P4 - SLOPE MINIMUM

P5 - SLOPE MAXIMUM

P6 - RETURN TO BASELINE

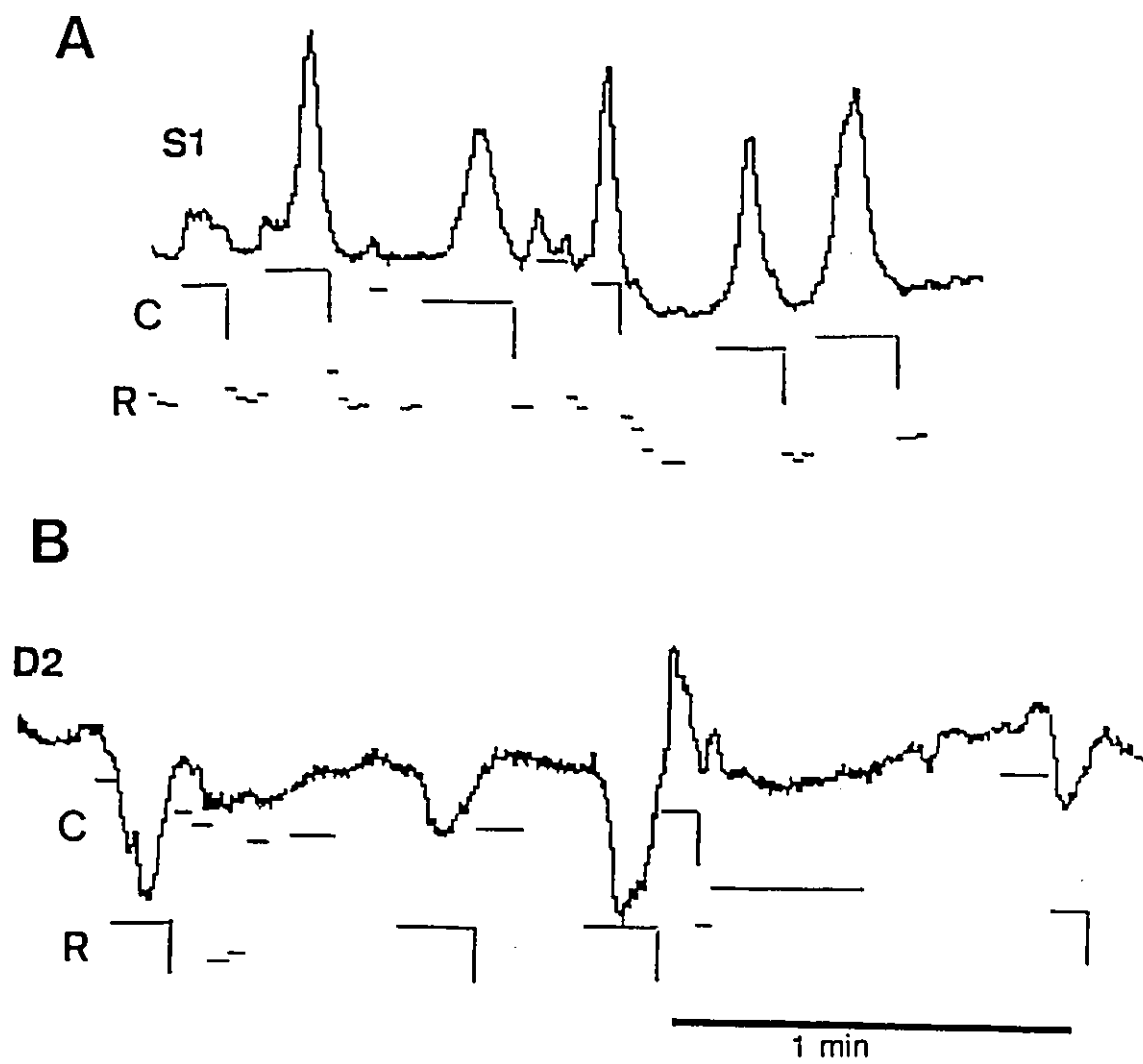
parameters N_1-N_6) were detected by the computer. In addition, detection of contractions and relaxations occurred simultaneously. For example, when a potential contraction was detected, the relaxation detector was inhibited.

Each of the six parameters was defined individually for each of the recording sites and stored in a parameter set within each data file. In order to ensure the most accurate determination of a contraction/relaxation minimum and maximum, the value of each parameter could be adjusted to match the type of response being recorded.

Figure 24 illustrates the marking of contractions and relaxations by the computer, using our modified method of Schemann et al (1986). For every channel in each experiment, the parameters (P_1-P_6 ; N_1-N_6) were fit to the data. Two series of horizontal and vertical lines were drawn below each contraction and relaxation in the computer-drawn motility tracings. Upon the detection of a contraction/relaxation, a horizontal line was drawn. A vertical line was added to the horizontally drawn line only when the recorded contraction/relaxation fulfilled all six previously defined parameters. This 'marking' of contractions and relaxations by the computer was determined to be very accurate.

The frequency, amplitude and duration of marked contractions and relaxations were calculated over 60 second periods and stored in ASCII files with the defined parameter set (P_1-P_6 ; N_1-N_6) and data channel number.

Figure 24. Typical computer-generated recordings taken from the a) antrum and b) duodenum (D2: 4cm distal to the pylorus) of a conscious rat, illustrating the detection of contractions and relaxations by the computer. A horizontal line connected to a vertical line depicts the detection of a genuine contraction (C) or relaxation (R).



3.2.1c Data Analysis.

Data analysis was performed using Quattro Pro (Version 3.0, Borland International) and Statgraphics Plus (Version 5.1, Statistical Graphics Corporation) software. Data was collated every 60 seconds, averaged over 10 minute periods, and expressed as a percentage of the first 3 hours of recording (control period) for each animal. Parameters analyzed included frequency, amplitude and duration of both contractile and relaxant motor activity. The area under the curve was also calculated which provided a measure of the amount of contractile/relaxant activity. Rats were then grouped according the parameter being analyzed and the treatment regimen. All quantitative results are expressed as the mean \pm SEM (standard error of the mean). A one-way analysis of variance (ANOVA) with the Tukey multiple comparison test was used to determine statistical significance between group data. A probability value of $p < 0.05$ was considered statistically significant.

3.3 In Vivo Motility Experiments.

3.3.1 Control Experiments

Control experiments were undertaken to determine the normal patterns of motility of the rat gastroduodenum, and consisted of two separate groups of

animals.

Group one: received no treatment, with motor activity recorded from the antrum (S1: 2cm proximal to the pylorus) and proximal duodenum (2cm (D1) and 4cm (D2) distal to the pylorus) for up to 9 hours.

Group two: received a subcutaneous injection of 0.9% saline at the time point of 3 hours, which was followed by up to six hours of continuous recordings. This allowed assessment of vehicle effects on motor activity. The data obtained from these studies were subsequently used for direct comparison against the cysteamine-HCl and drug treated rats.

3.3.1a Antrum

Contractions and relaxations were recorded as shown in the typical recording in Figure 25. Motor activity was cyclical, with both propagating and local, non-propagating activity observed (Figure 25a and b). This motor activity was not affected with time, or by saline injection as shown in Figure 26.

The analysis software did not allow quantification of the relative amount of contractile motor activity to relaxant motor activity. However, qualitatively, contractile activity appeared to predominate, but was not necessarily of greater magnitude, in terms of amplitude, duration or area, than relaxant activity.

Figure 25. Original recordings illustrating a) propagating and b) local, non-propagating motor activity of the antrum (S1) and duodenum (D1 and D2) of the rat under normal, control conditions. The hatched lines indicate propagating motor responses of the gastroduodenum. The time bar represents 1 minute.

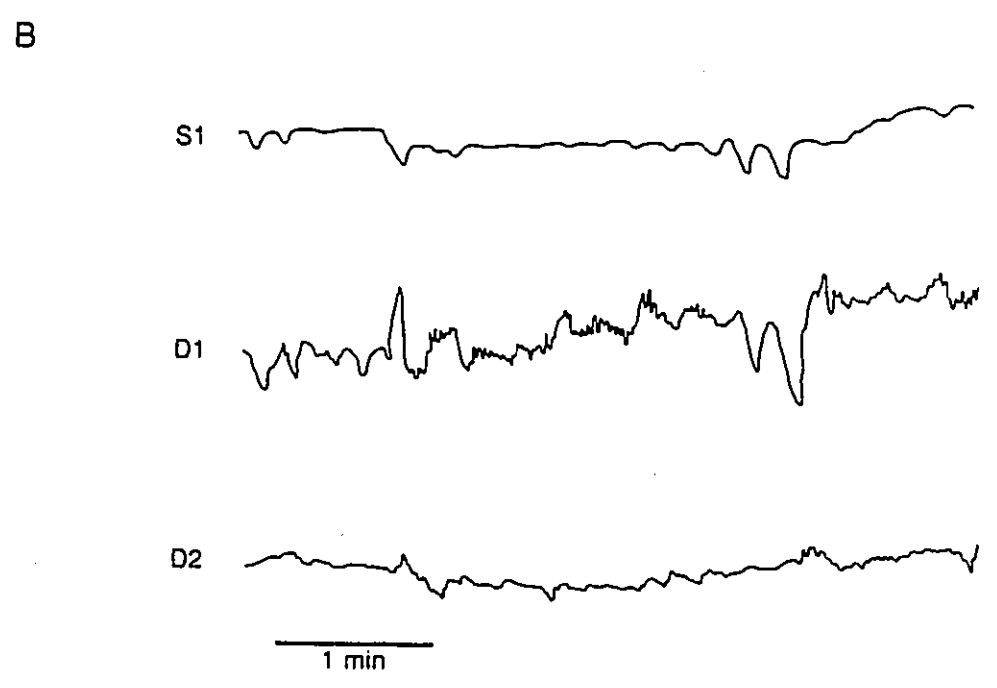
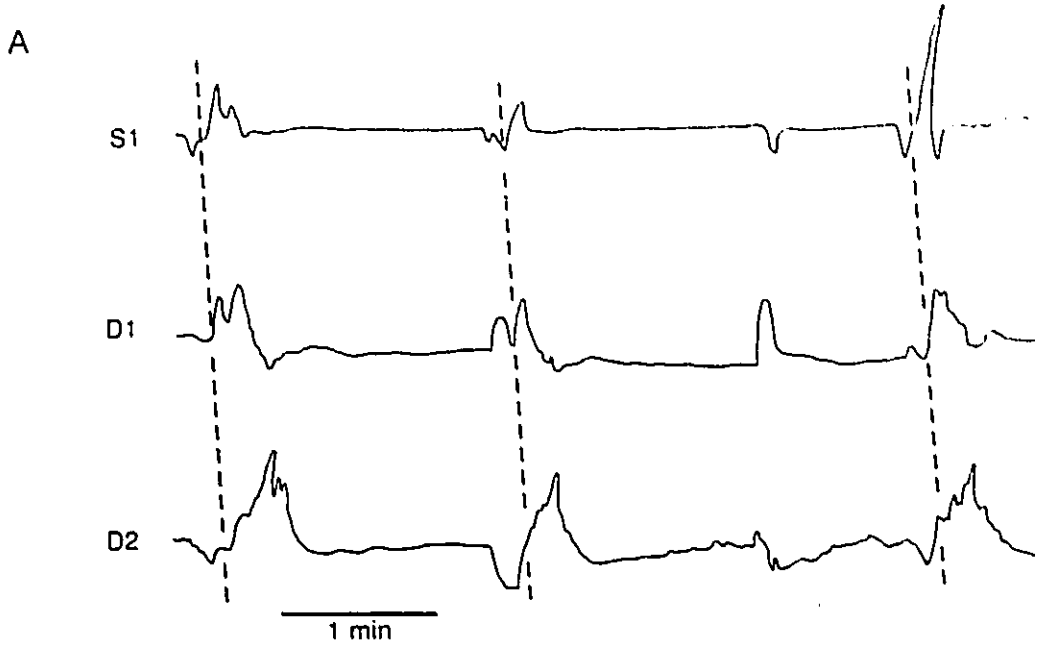
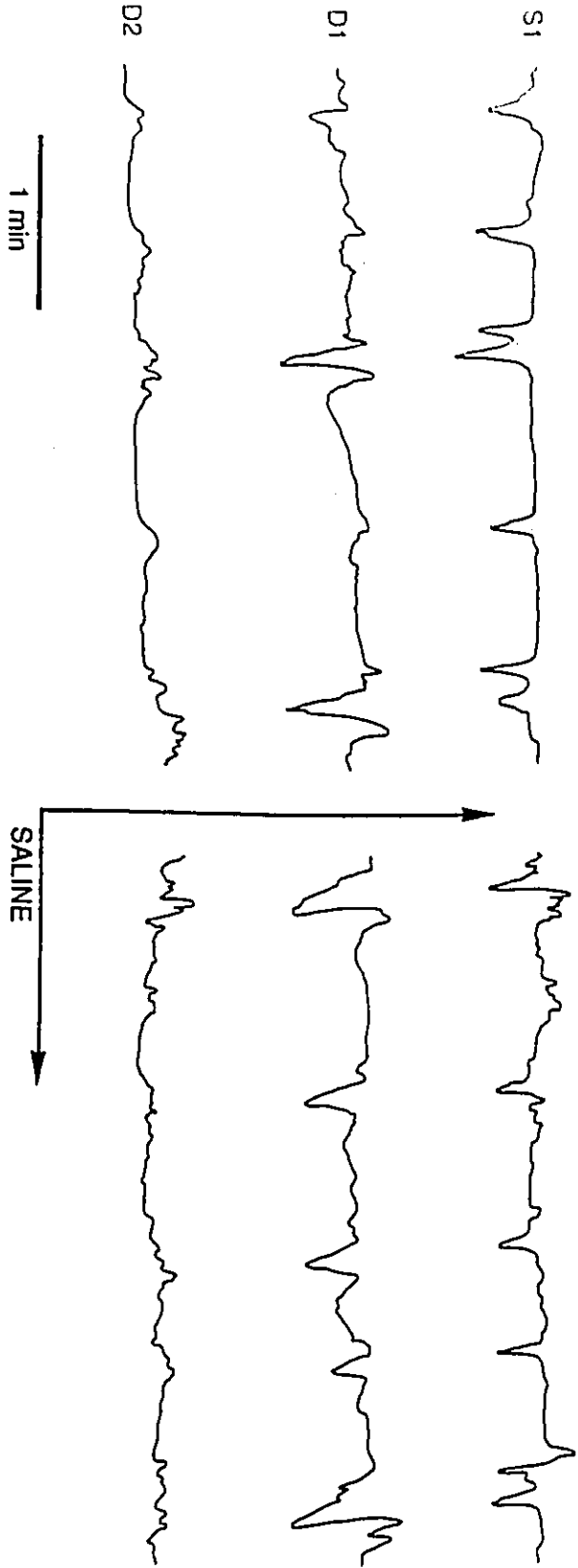


Figure 26. Original recordings showing the effects of saline on motor activity of the antrum (S1) and duodenum (D1 and D2) in the conscious, unrestrained rat. Saline (0.9%) was administered sc immediately following the 3 hour control recording period. The time bar represents 1 minute.



Antral Contractions

The frequency, amplitude and duration of antral contractions in non-treated (n=2) and saline-injected (n=2) control animals is summarized in Figure 27a-c. In addition, the 'area' of contraction, which is used as a measure of the total amount of contractile activity, is presented in Figure 27d. No significant differences ($p>0.05$) in the measured parameters were evident between the two groups of control animals. There was also no significant effect of time on antral contractility within or between non-treated and saline-treated control animals (Figure 28a-d).

Antral Relaxations

As illustrated in Figure 29a-d, the frequency, amplitude, duration and total amount (area) of antral relaxant activity in saline-injected animals (n=2) was not significantly different ($p>0.05$) from non-treated control animals (n=3). Similarly, there was no significant alteration in the profile of the measured parameters within or between the control groups (Figure 30a-d).

3.3.1b Duodenum - D1

Both contractions and relaxations were evident at the D1 recording site (Figure 25). This included motor activity originating in the antrum (S1) and

Figure 27. Effects of saline on the a) frequency; b) amplitude; c) duration and d) amount of contractile activity in the rat antrum. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Data was collated for every minute, averaged over 10 minute periods and expressed as a percentage of the mean of the first 3 hours of recording. Animals were then grouped according to the treatment regimens, with the mean \pm SEM presented. The number (n) of animals for the different treatment groups is given in the abscissa.

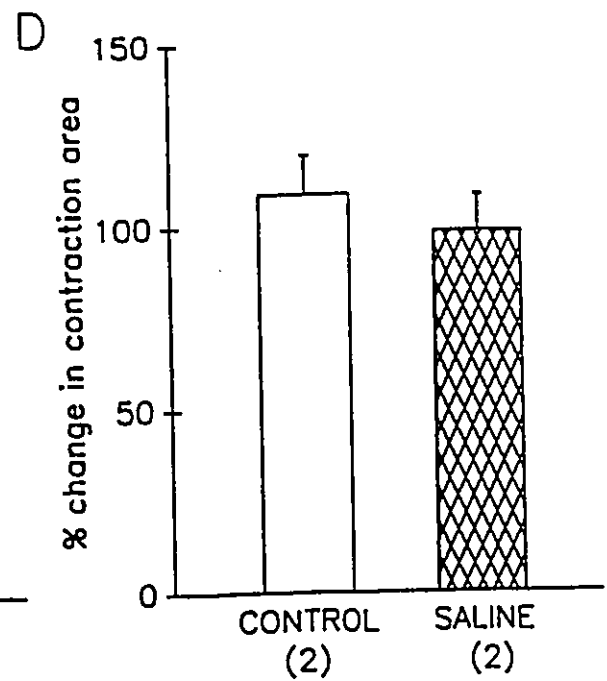
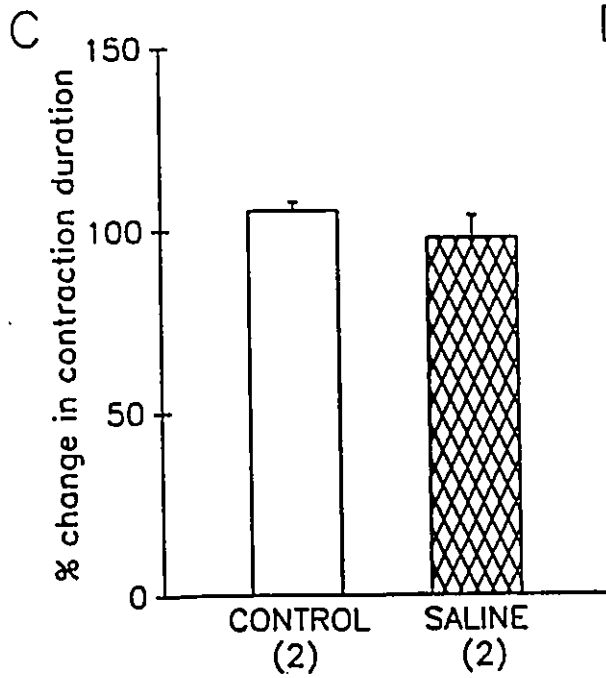
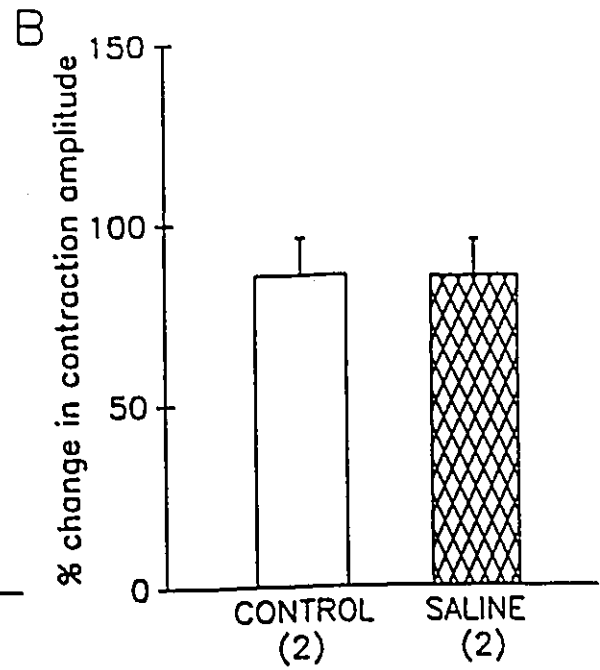
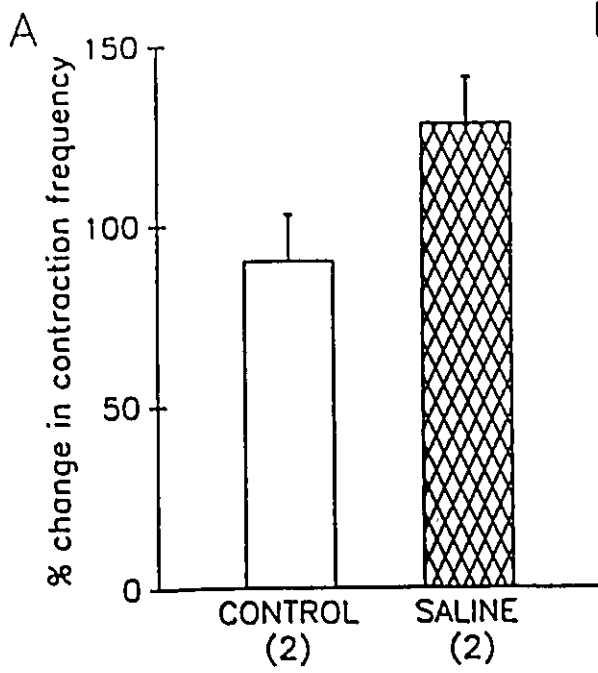


Figure 28. Time-course of changes in the a) frequency; b) amplitude; c) duration and d) amount of contractile activity in the rat antrum following the administration of saline. Data is presented as the mean \pm SEM for each group at the time points indicated in the abscissa. n represents the number of rats.

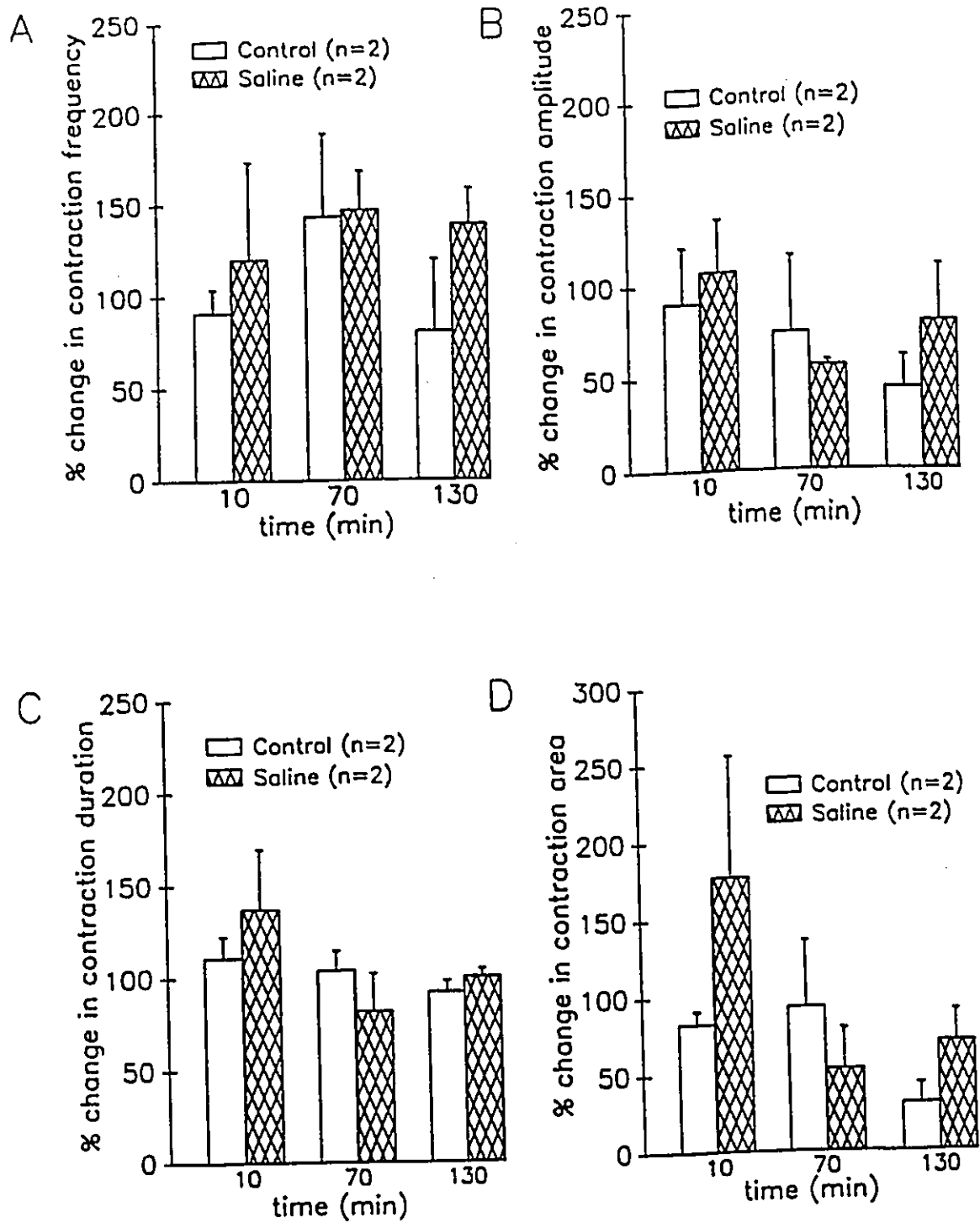


Figure 29. Effects of saline on the a) frequency; b) amplitude; c) duration and d) amount of relaxations in the rat antrum. The amount of relaxant activity was determined from the area under the curve, and is presented as 'area'. Data was collated for each minute, averaged over 10 minute periods and expressed as a percentage of the mean of the first 3 hours of recording. Animals were then grouped according to the given treatment, with the mean \pm SEM presented. The number (n) of animals for the various treatment groups is given in the abscissa.

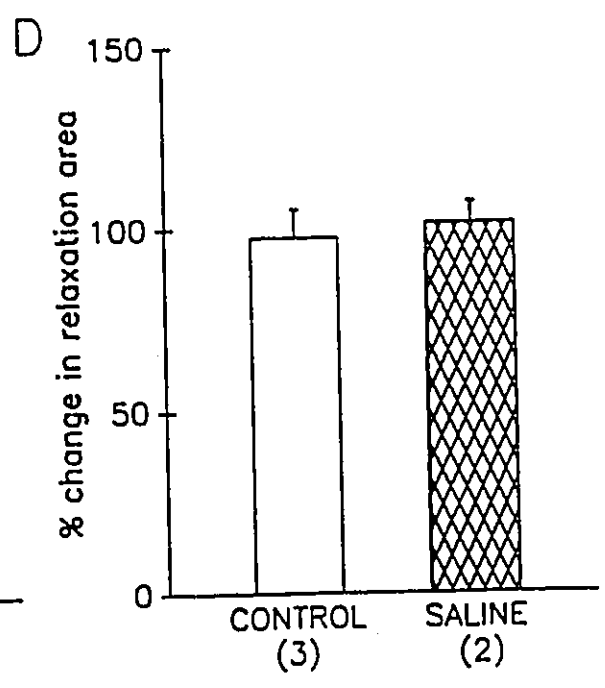
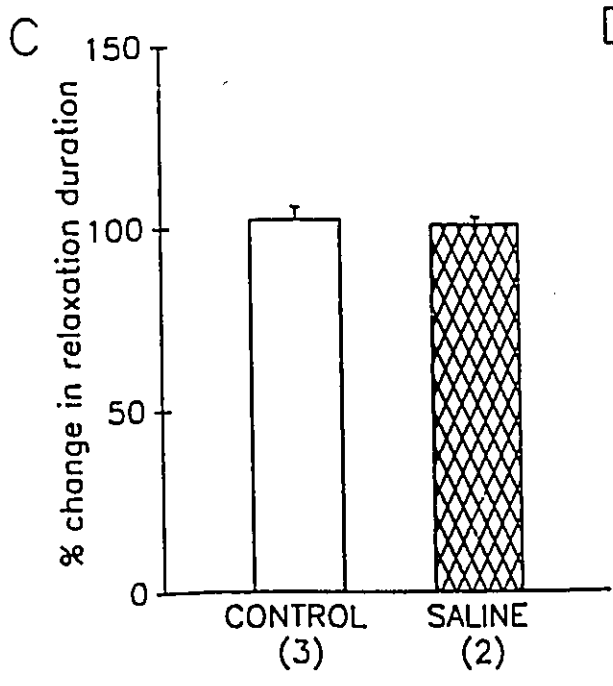
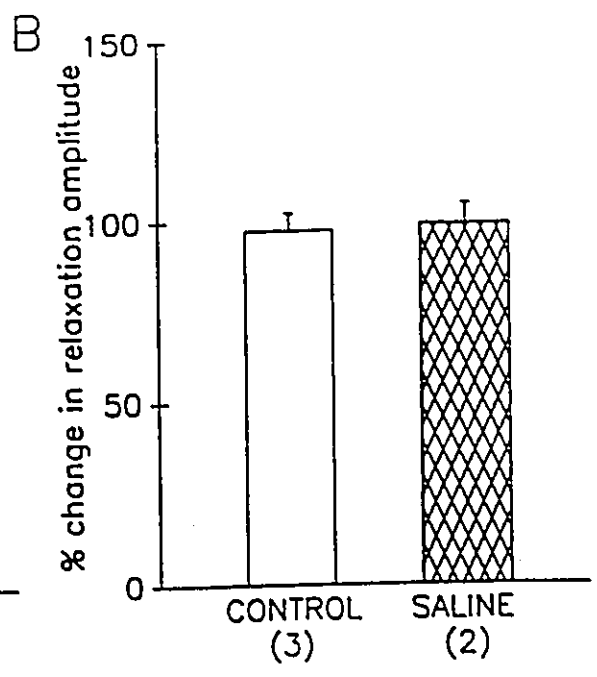
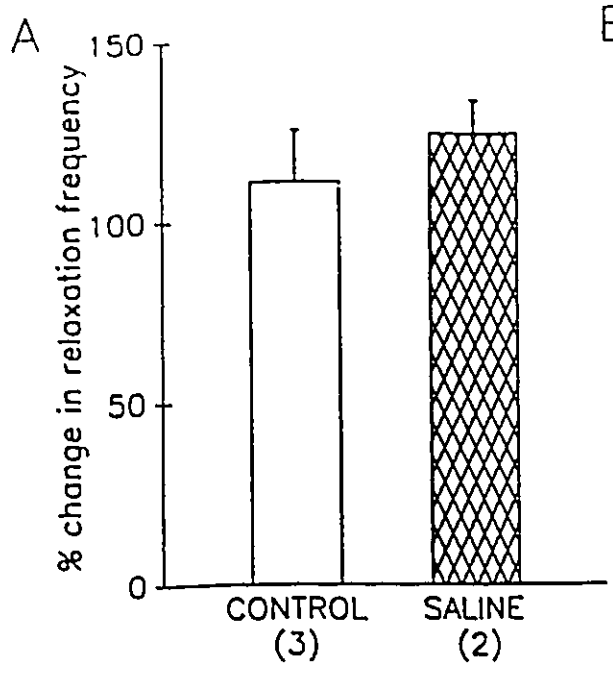
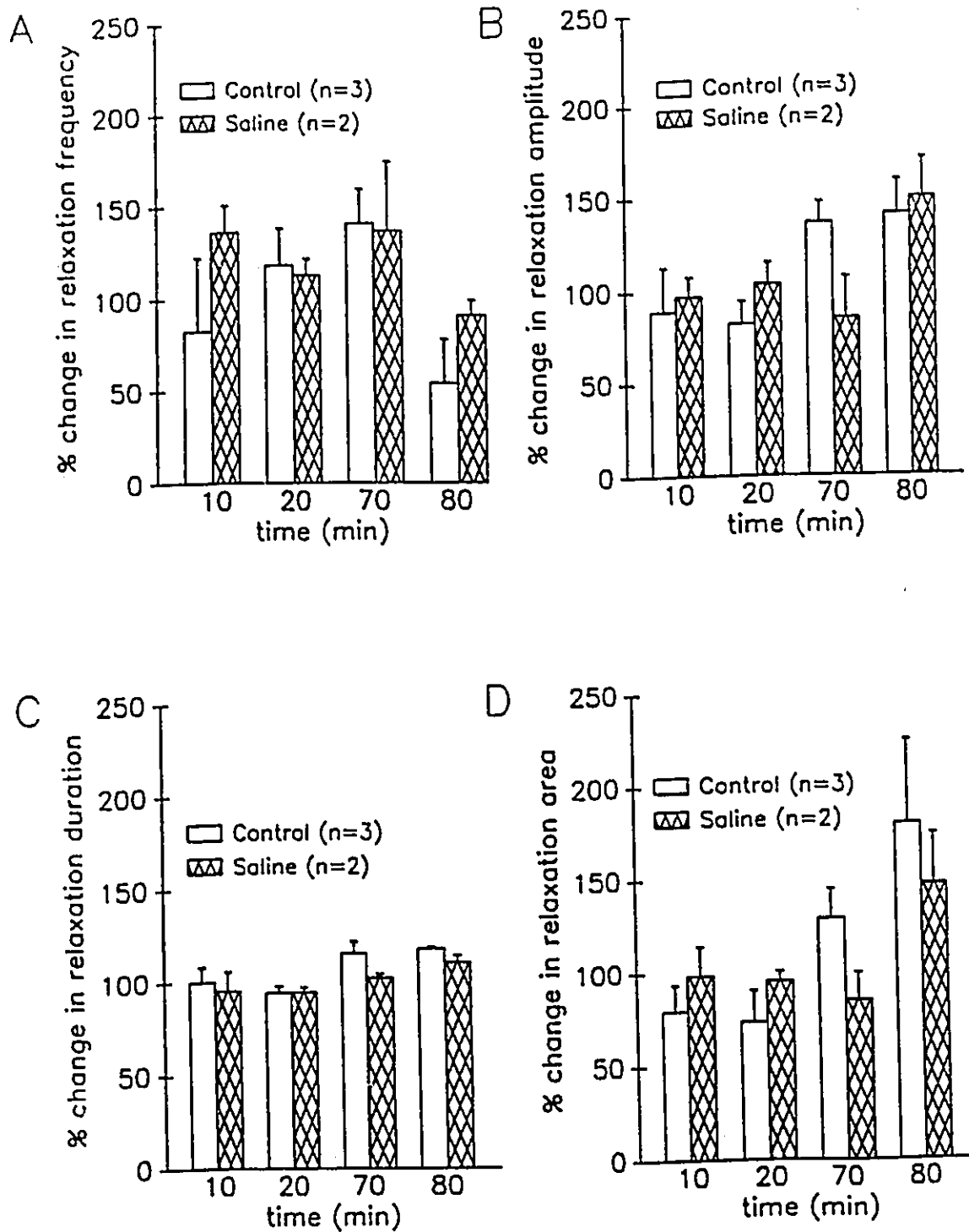


Figure 30. Time-course of changes in the a) frequency; b) amplitude; c) duration and d) amount of relaxant activity in the rat antrum following the administration of saline. Data is presented as the mean \pm SEM for each treatment group at the time points indicated in the abscissa. n represents the number of rats used.



propagating to D1, with some of these motor events continuing along the duodenum to D2, as presented in Figure 25a. Local, non-propagating motor activity was also recorded (Figure 25b). These motor activities were not affected by time.

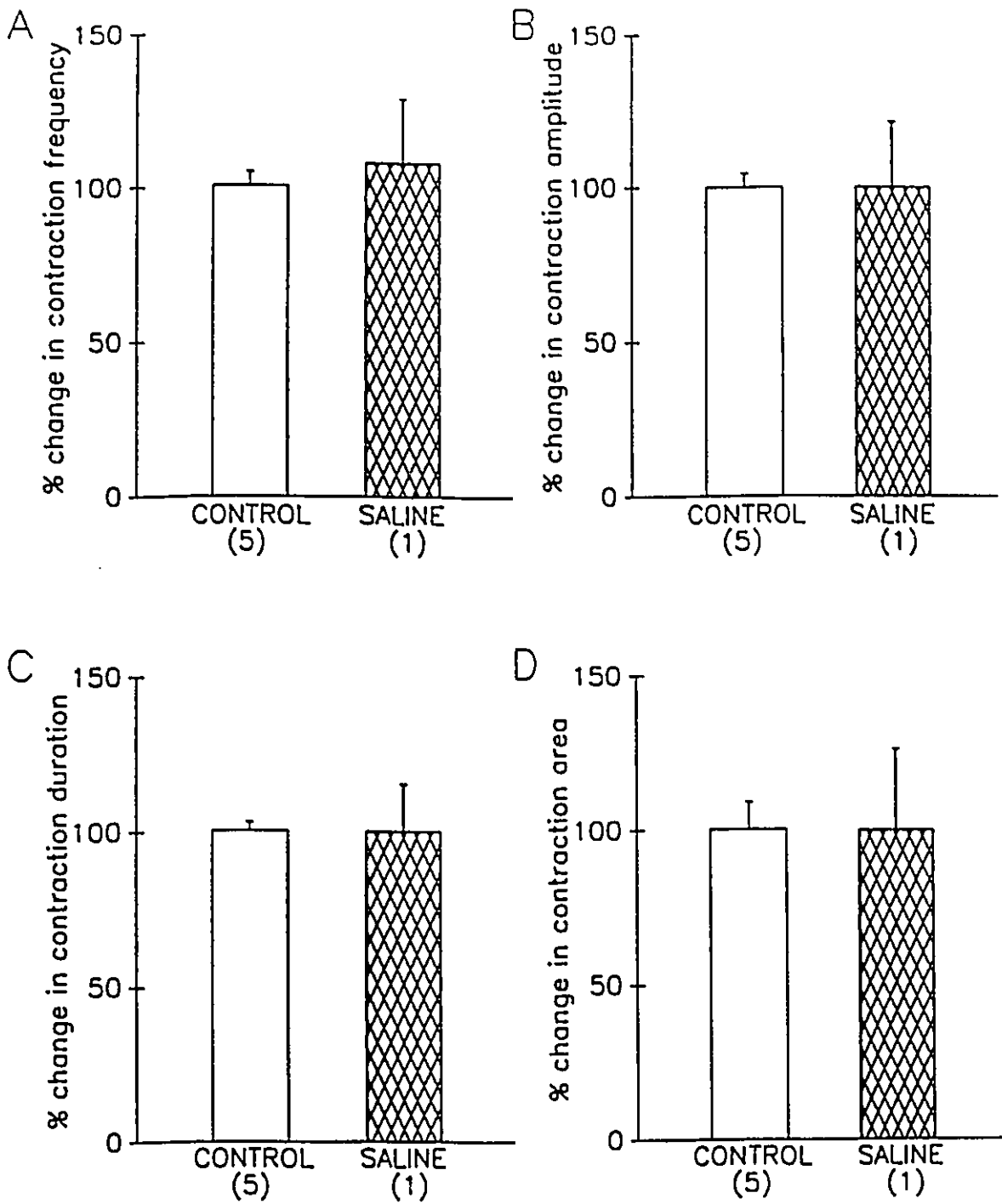
Relaxations were recorded from the proximal duodenum, but contractions were observed to be the predominating motor event. Although contractile motor activity was observed with greater frequency, these responses were not necessarily of greater magnitude, in terms of amplitude, duration or area.

The administration of saline did not appear to affect duodenal motility, as shown in the real-time tracing in Figure 26.

Contractions at D1

Changes in frequency, amplitude and duration of duodenal (D1) contractile activity in non-treated and saline-injected control animals are summarized in Figure 31a-c. The total amount of contractile activity is shown in Figure 31d. Due to technical problems associated with the D1 recording site, data from only one of four saline-injected (Group 2) animals could be used for statistical analysis. When compared to the non-treated (Group 1) control animals (n=5), the saline-injected rat showed no changes in duodenal contractility. Although the motility patterns of the remaining three rats could not be computer analyzed, comparison of the real time tracings showed no changes in the contractions following the injection of saline.

Figure 31. Effects of saline on the a) frequency; b) amplitude; c) duration and d) amount of contractile activity recorded at the D1 site (2cm distal to the pylorus) of the rat proximal duodenum. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Data was collated for every minute, averaged over 10 minute periods and expressed as a percentage of the mean of the first 3 hours of recordings. Animals were then grouped according to the treatment regimens, with the mean \pm SEM presented. The number (n) of animals for the different treatment groups is given in the abscissa.



Relaxations at D1

Although relaxations were observed at the duodenal D1 recording site, there were not enough relaxations per animal over equivalent time periods to allow for valid statistical comparison between the two groups of controls. On a purely qualitative basis, the saline injection did not appear to cause any changes to the relaxation responses.

3.3.1c Duodenum - D2

Motor activity obtained from the D2 site consisted of both contractions and relaxations, with propagating and local, non-propagating motor activity observed (Figure 25). Time did not affect these motor events.

Similar to that observed in both the stomach and early proximal duodenum (D1), contractions predominated over relaxations. However, in terms of amplitude, duration and area, individual contractions were not necessarily larger than individual relaxations.

Contractions at D2

The injection of saline caused no apparent disruption to the motor activity at D2 as shown in the real-time tracing in Figure 26. The frequency, amplitude,

duration and total amount (area) of contractile activity measured at D2 in non-treated (n=3) and saline-injected (n=2) animals are summarized in Figure 32. There were no significant ($p>0.05$) differences between the two groups of control animals. In addition, there were no significant differences in the measured parameters with time within or between the non-treated and saline-injected control rats (Figure 33a-d).

Relaxations at D2

Although relaxations were evident at the D2 recording site, the number of relaxations observed per animal were too low to permit statistical analysis. However, from the real time tracings there did not appear to be any alterations in the relaxation activity following the saline injection.

3.3.1d Summary.

Under control conditions, both contractions and relaxations were recorded from the gastroduodenum of conscious, unrestrained rats. This is the first in vivo study to record and analyze both contractions and relaxations from the mammalian gastroduodenum. Previous in vivo studies, using a vast array of recording techniques, have only been able to demonstrate smooth muscle contractions. However, motility is a complex, coordinated process, involving both smooth muscle

Figure 32. Effects of saline on the a) frequency; b) amplitude; c) duration and d) amount of contractile activity recorded at the D2 site (4cm distal to the pylorus) of the rat duodenum. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Data was collated for every minute, averaged over 10 minute periods and expressed as a percentage of the mean of the first 3 hours of recording. Animals were grouped according to the treatment regimens, with the mean \pm SEM presented. The number (n) of animals for the different treatment groups is given in the abscissa.

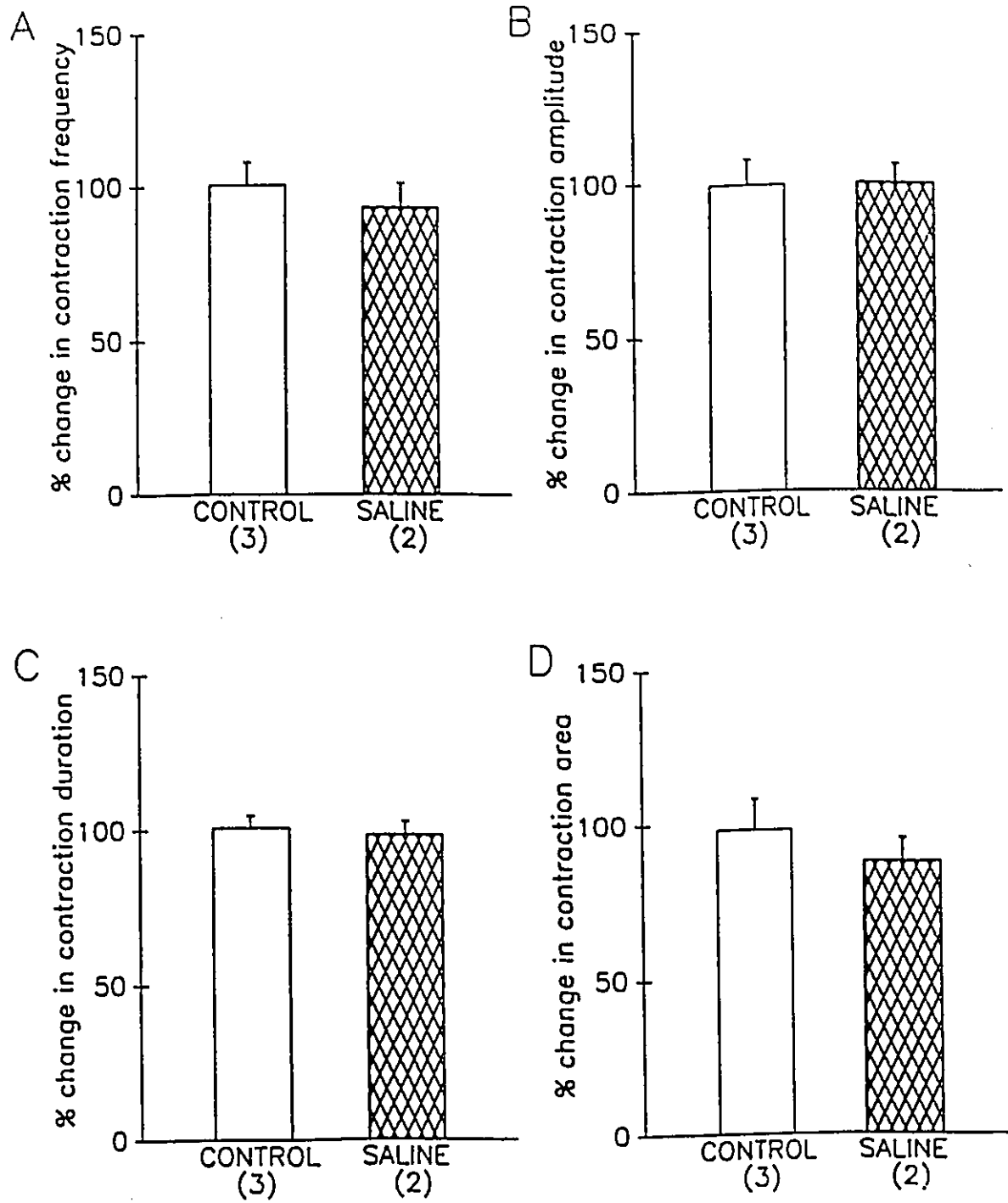
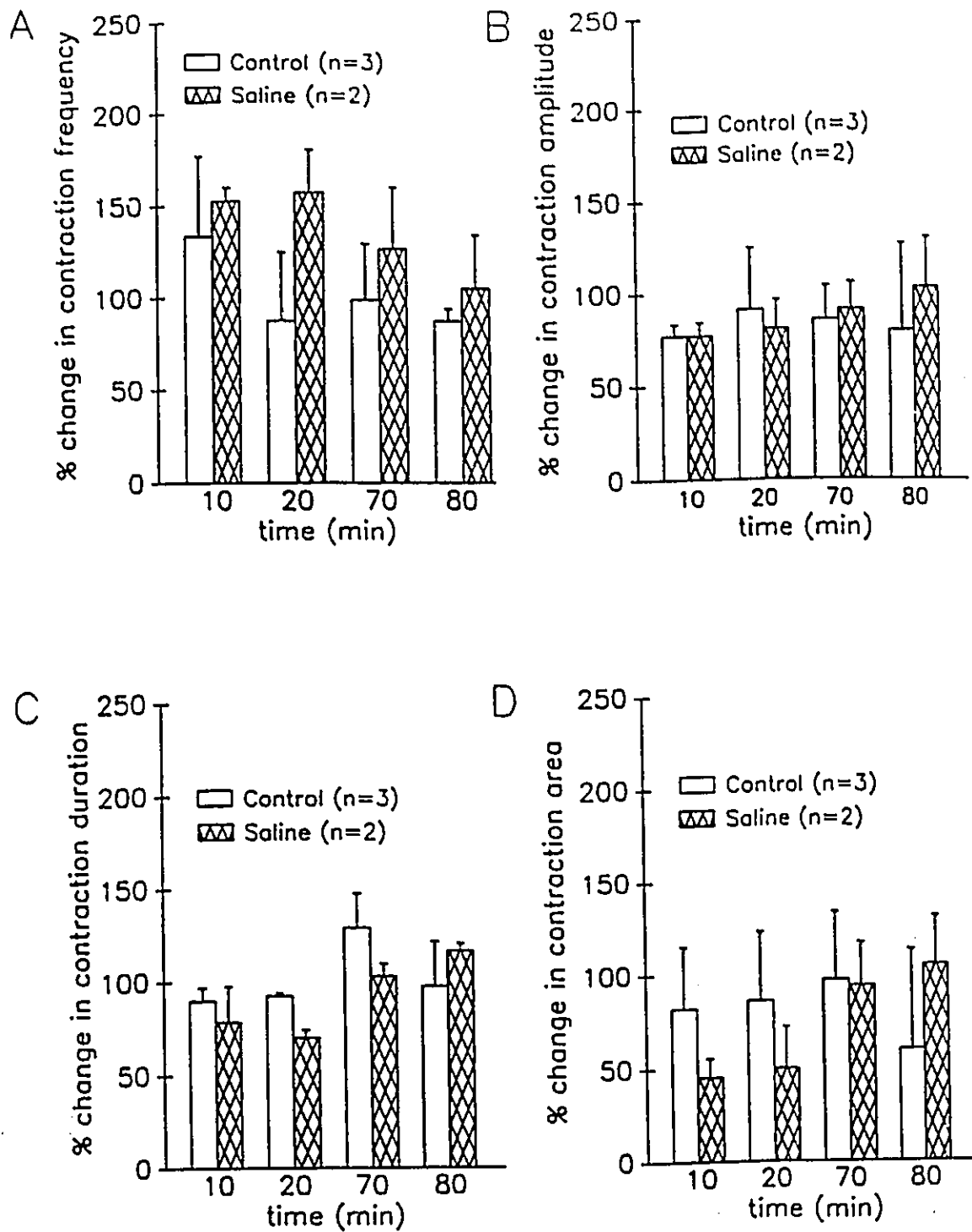


Figure 33. Time-course of changes in the a) frequency; b) amplitude; c) duration and d) amount of contractile activity in the rat duodenum (D2) following the administration of saline. Data is presented as the mean \pm SEM for each treatment group at the time points indicated in the abscissa. n represents the number of rats used.



contractions and relaxations. At all three recording sites (S1, D1 and D2), contractile motor activity was observed to predominate over smooth muscle relaxations. However, in terms of amplitude, duration and area of these responses, contractions were not necessarily of different magnitude to that of the relaxations. It is not surprising to find that contractile activity was more frequently observed when considering the function of the gastroduodenum. Although both contractions and relaxations are required for normal peristalsis and anally-directed movement of intestinal contents, contractions also function in the mixing and circulation of ingested food contents with digestive secretions and enzymes, and participate in migrating motor complex activity. In addition, propagating and local non-propagating motor activity was observed, indicative of the animal's fasted state.

During control conditions, the four phases of the migrating motor complex (MMC) could not be distinguished at any of the recording (S1, D1, D2) sites. However, Wittman et al (1990) have provided evidence that stress can disrupt the recurrence of MMCs in rats. This may explain the lack of typical MMC type activity in the present study. Another possibility is that ileus prevented the visualization of MMCs (Dr. R.B. Scott, personal communication). However, 48 hours following the surgical procedure, motility recordings could not be obtained due to the presence of adhesions which prevented the proper balancing of the strain gauges. It has also been proposed (Dr. Greenwood, Lily Drugs, personal communication) that the close positioning of the recording devices on the intestine may prevent visual

observation of MMC activity.

Treatment with saline did not significantly alter antral or duodenal (D1 and D2) contractile and relaxant motor activity. Since there were no statistical differences in antral or duodenal (D2) motor activity between the non-treated (Group 1) and saline-injected (Group 2) control groups respectively, data from these rats were pooled (according to the specific region) for subsequent statistical comparisons. However, at the duodenal D1 recording site, data is only presented from the Group 1 control animals since technical problems were associated with three of the four Group 2 saline-treated rats, and thus, precluded data analysis.

3.3.2 Effects of Cysteamine-HCl on Gastroduodenal Motility.

The purpose of these experiments was to determine the effect of the ulcerogen cysteamine-HCl on motor activity of the rat gastroduodenum.

Cysteamine-HCl (10-100mg/100g) can be administered by subcutaneous injection or by oral gavage to induce duodenal ulceration in Sprague-Dawley rats (Robert et al, 1974; Fujii and Ishii, 1975; Szabo et al, 1978). In this study, cysteamine-HCl was first tested as a single intragastric bolus of 56mg/100g. However, this route of administration resulted in complete disruption of the recording. This appeared to be due to the distension-induced stimulation of the gut wall. In addition, oral gavage of cysteamine-HCl required considerable handling of the rat, which increased the chances of damage to the strain gauge

leads.

In subsequent experiments, cysteamine-HCl was given by subcutaneous injection in the neck region. However, a single bolus injection of cysteamine-HCl (56mg/100g), resulted in discomfort to the rat. Therefore, the administration protocol was changed, so that cysteamine-HCl was given in two doses of 28mg/100g sc, one hour apart. This drug protocol was considerably less distressing to the animals, and thus, was chosen for the remaining experiments.

Following a three hour control recording period, cysteamine-HCl was administered and motility was recorded for up to six hours. In many experiments, it was difficult to obtain a complete six hour recording of gastroduodenal motility due to the rats experiencing convulsions following the cysteamine-HCl treatment. Convulsions in cysteamine-HCl treated rats usually occurred within four hours of the ulcerogen injection. This is not a unique finding since Szabo et al (1987) observed that cysteamine-HCl (90mg/100g po) caused excitation and stereotypic movements, which often progressed to convulsions and death of the rat.

The effects of subcutaneous administration of cysteamine-HCl (2x28mg/100g) on antral and duodenal motility are described below.

3.3.2a Antrum

During the initial three hour control recording period, both contractions and relaxations were usually recorded, with no apparent differences in magnitude

between these two types of responses. However, contractile activity predominated over relaxations.

Cysteamine-HCl immediately disrupted antral motility, abolishing cyclical, patterned motor activity for up to six hours (Figure 34). In fact, cysteamine-HCl enhanced contractile motor activity, establishing a basal-like pattern of repeating contractions while relaxations were rarely observed. Thus, quantitative analysis could only be performed on contractile motor responses following cysteamine-HCl treatment.

Antral Contractions

Cysteamine-HCl treatment increased all measured parameters of antral contractility (Figure 35).

Contraction frequency was increased ($93 \pm 25\%$, $p < 0.05$) compared to the three hour pre-cysteamine-HCl control period. When compared to control animals ($n=4$), the cysteamine-HCl treatment ($n=5$) increased contraction frequency $85 \pm 12\%$ ($p < 0.05$) as summarized in Figure 35a.

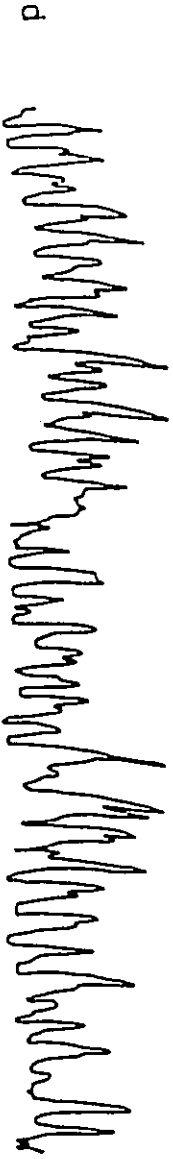
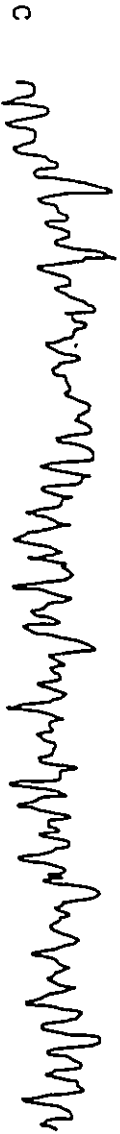
Changes in the amplitude of contractile activity in response to cysteamine-HCl are summarized in Figure 35b. Cysteamine-HCl treated animals demonstrated no significant change ($p > 0.05$) in contraction amplitude compared to control animals ($n=4$). However, three of five cysteamine-HCl treated rats showed a significant increase ($89 \pm 21\%$, $p < 0.05$) compared to the three hour pre-

Figure 34. Original recordings showing the effects of cysteamine-HCl over time on antral motility in the conscious, unrestrained rat at selected time points. a) control; b) 0-7 minutes post-cysteamine-HCl; c) 70-77 minutes post-cysteamine-HCl and d) 165-172 minutes post-cysteamine-HCl. Cysteamine-HCl (2x28mg/100g sc) was administered in 2 boluses, 1 hour apart, immediately following the 3 hour control recording period. Time bar represents 1 minute.

CONTROL

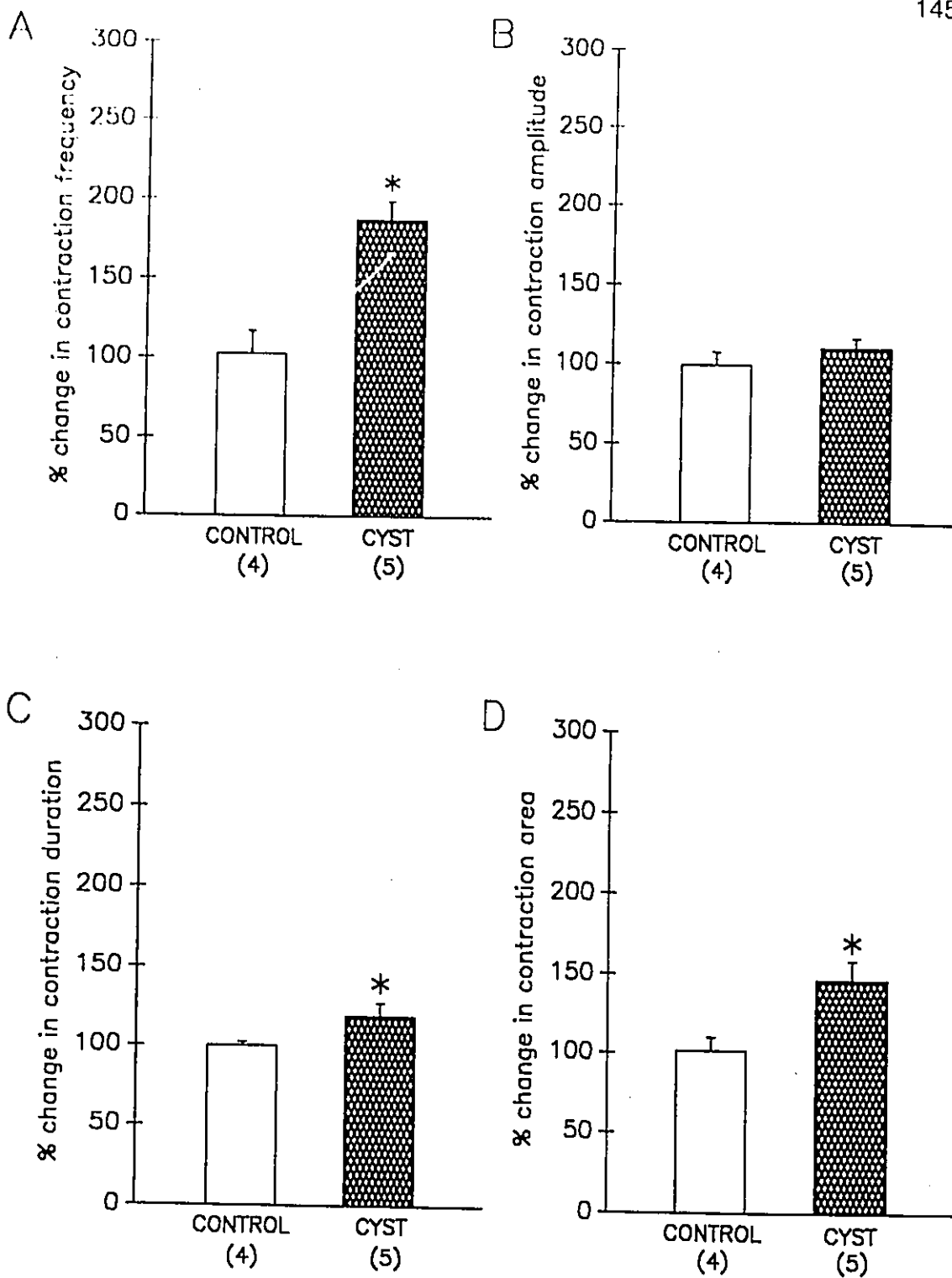


CYSTEAMINE



1 min

Figure 35. Comparison of the a) frequency; b) amplitude; c) duration and d) amount of antral contractile activity between control and cysteamine-HCl treated rats. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Cysteamine-HCl (CYST) was given in 2 doses of 28mg/100g sc. Data is presented in columns as the mean \pm SEM. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control group. The number (n) of rats for the different treatment groups is given in the abscissa.



cysteamine-HCl control period and a $44 \pm 9\%$ ($p < 0.05$) increase over control rats.

A significant increase in contraction duration ($19 \pm 5\%$, $p < 0.05$, Figure 35c) occurred in cysteamine-HCl treated rats ($n=5$) compared to control animals ($n=4$). Within the cysteamine-HCl treated group, three of five rats showed increases ($p < 0.05$) in contraction duration from 38% to 81% compared to the pre-cysteamine-HCl control period.

Total contractile activity was increased ($46 \pm 12\%$, $p < 0.05$, Figure 35d) in cysteamine-HCl treated animals ($n=5$) compared to control rats ($n=4$). In fact, 60% of cysteamine-HCl treated rats demonstrated a significant increase ($p < 0.05$) in contraction area compared to the pre-cysteamine-HCl control period ($130 \pm 24\%$) and control animals ($137 \pm 15\%$).

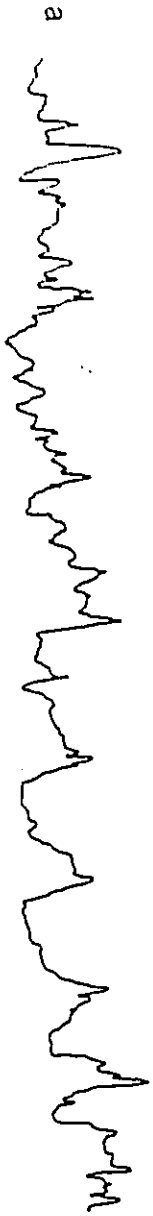
3.3.2b Duodenum - D1

During the control recording period, both contractions and relaxations were present, with the predominating motor event being contractions.

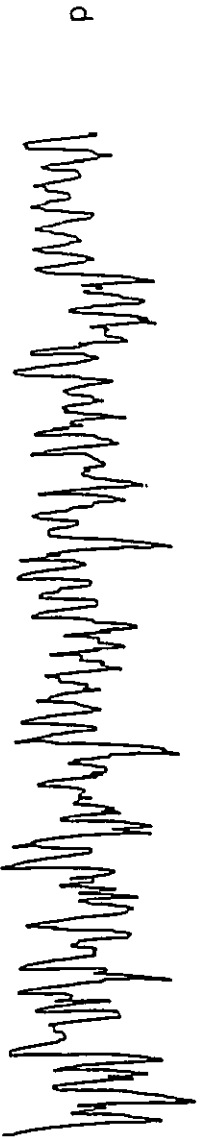
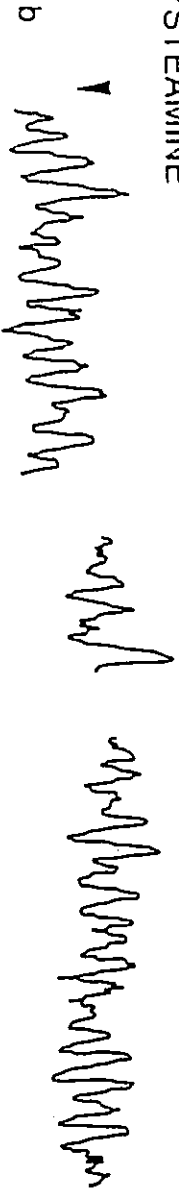
Cysteamine-HCl immediately abolished normal local and propagating motor activity (Figure 36). In many instances, cysteamine-HCl induced a pattern of repetitive contractile activity which persisted for up to 6 hours. Similar to the antrum, relaxations were only occasionally observed following the cysteamine-HCl treatment, and are not discussed due to the low number recorded per animal.

Figure 36. Original recordings showing the effects of cysteamine-HCl over time on proximal duodenal (D1) motility in the conscious, unrestrained rat at selected time points. a) control; b) 0-7 minutes post-cysteamine-HCl; c) 120-127 minutes post-cysteamine-HCl and d) 150-157 minutes post-cysteamine-HCl. Cysteamine-HCl (2x28mg/100g sc) was given in 2 boluses, 1 hour apart, immediately following the 3 hour control recording period. Spaces in the recording in (b) represent artefactual responses to movement of the animal which have been deleted. Time bar represents 1 minute.

CONTROL



CYSTEAMINE



1 min

Contractions at D1

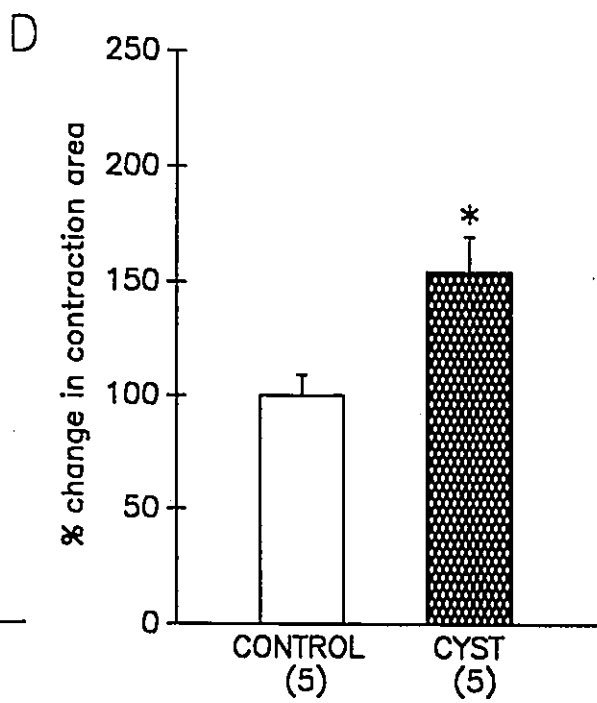
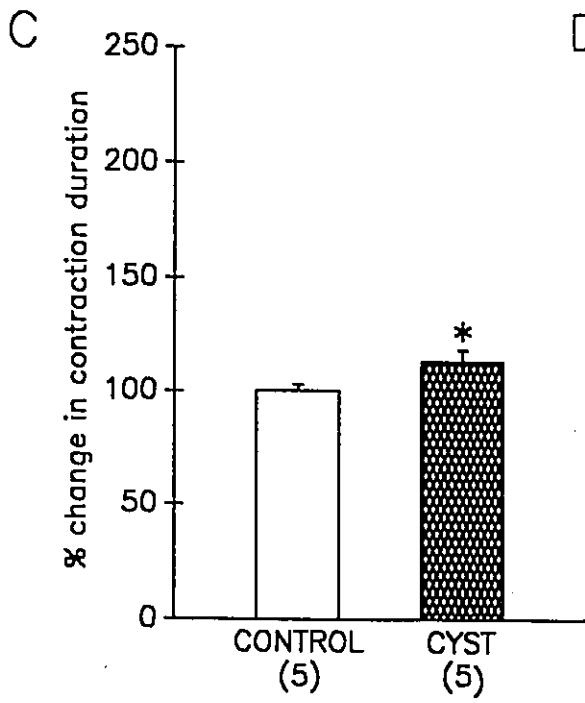
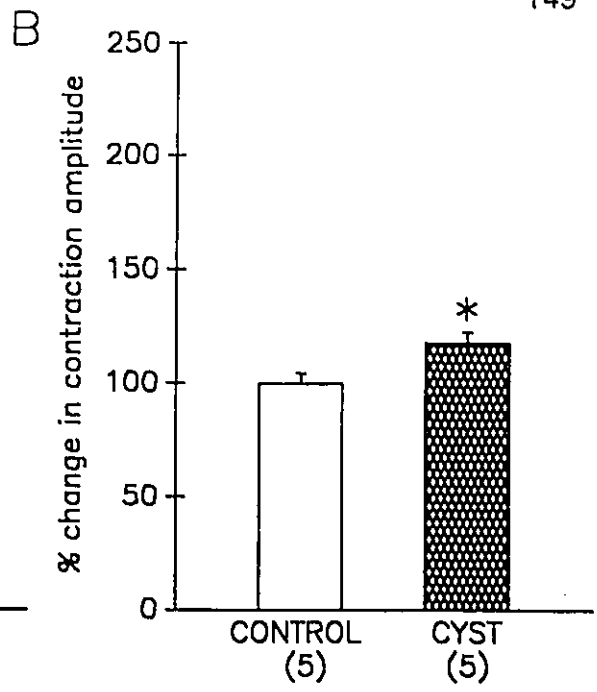
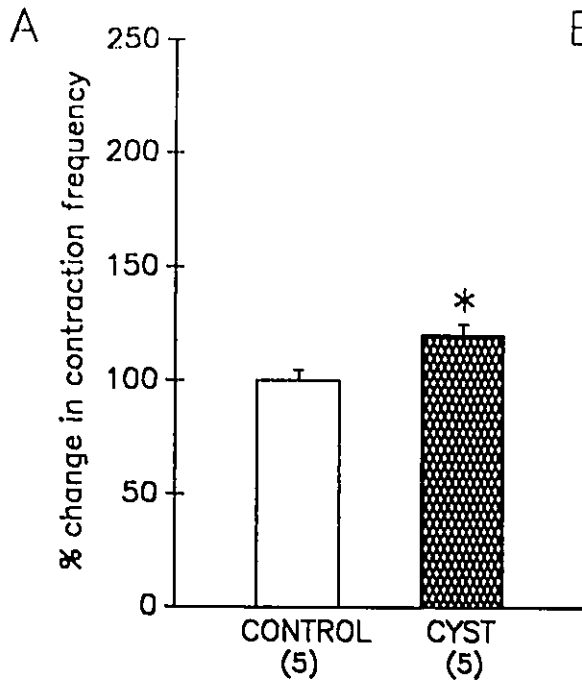
Contraction frequency was significantly increased ($20 \pm 5\%$, $p < 0.05$, Figure 37a) in cysteamine-HCl treated rats ($n=5$) compared to control animals ($n=5$). However, within the cysteamine-HCl treated group, only three of five rats showed significant increases in contraction frequency compared to both the 3 hour pre-cysteamine-HCl control period ($30 \pm 4\%$, $p < 0.05$) and control animals ($34 \pm 5\%$, $n=5$, $p < 0.05$).

In rats ($n=5$) receiving cysteamine-HCl treatment, no significant change in contraction amplitude was observed compared to the pre-cysteamine-HCl recording period. However, in three of these animals, contraction amplitude was increased by more than 18% following the administration of the ulcerogen. When compared to control rats ($n=5$), cysteamine-HCl treated rats demonstrated a significant increase ($18 \pm 5\%$, $p < 0.05$) in contraction amplitude (Figure 37b).

Contraction duration was significantly increased ($13 \pm 5\%$, $p < 0.05$, Figure 37c) in cysteamine-HCl treated rats ($n=5$) compared to control rats ($n=5$). However, no significant difference ($p > 0.05$) in contraction duration was measured in three of five cysteamine-HCl treated rats compared to the pre-cysteamine-HCl period. However, in two rats, the duration of contractions increased by 15% and 81% respectively.

The overall amount of contractility was significantly increased ($54 \pm 15\%$, $p < 0.05$, Figure 37d) in cysteamine-HCl treated rats ($n=5$) compared to the control

Figure 37. Comparison of the a) frequency; b) amplitude; c) duration and d) amount of duodenal contractile activity recorded at D1 (2cm distal to the pylorus) between control and cysteamine-HCl treated rats. The amount of contractile activity was determined from the area under the curve, and is presented as 'area'. Cysteamine-HCl (CYST) was administered in 2 doses (28mg/100g sc). Data is given as the mean \pm SEM. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control group. The number (n) of animals for each treatment group is given in the abscissa.



animals (n=5). However, two of five cysteamine-HCl treated rats showed striking increases of 162% and 373% in the amount of contractile activity whereas the remaining rats showed no significant change ($p>0.05$) compared to the control group.

3.3.2c Duodenum - D2

During the control recording period, predominantly contractile motor activity was observed at this recording site.

In contrast to the effects of cysteamine-HCl on motility at D1, normal motility at D2 was not completely disrupted following the administration of the ulcerogen (Figure 38). However, fewer relaxations were observed following cysteamine-HCl treatment, and therefore, statistical analysis of this type of motor activity was not possible.

Contractions at D2

Cysteamine-HCl treatment (n=3) had no significant effect ($p>0.05$) on contraction frequency compared to the three hour control period. However, when compared to control animals, a small but significant increase in duodenal contraction frequency ($19 \pm 5\%$, $p<0.05$) was evident, Figure 39a. This apparent contradiction suggests that the mean frequency of contraction in control animals

Figure 38. Original recordings showing the effects of cysteamine-HCl over time on duodenal (D2) motility in the conscious, unrestrained rat at selected time points. a) control; b) 0-4 minutes post-cysteamine-HCl; c) 35-39 minutes post-cysteamine-HCl and d) 110-114 minutes post-cysteamine-HCl. Cysteamine-HCl (56mg/100g sc) was given immediately following the 3 hour control recording period. Time bar represents 1 minute.

CONTROL

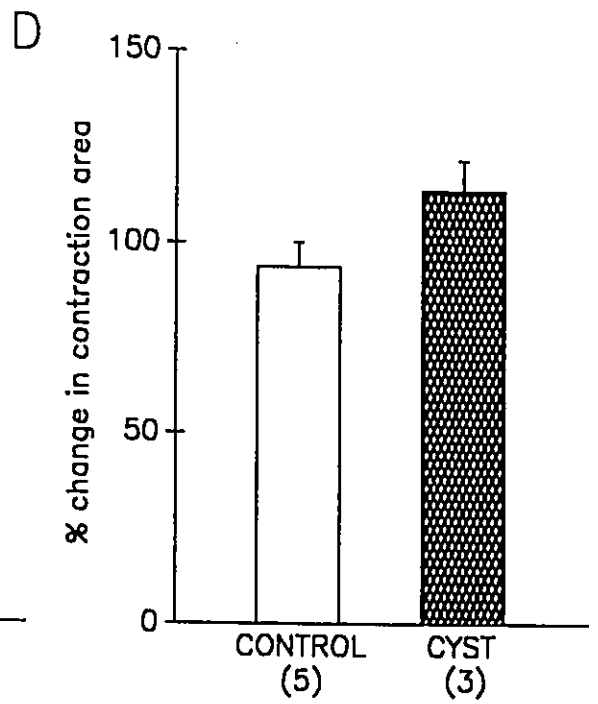
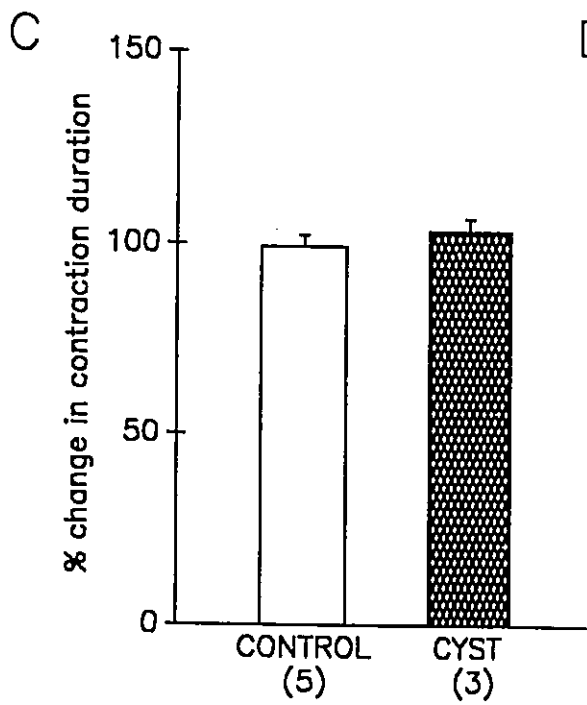
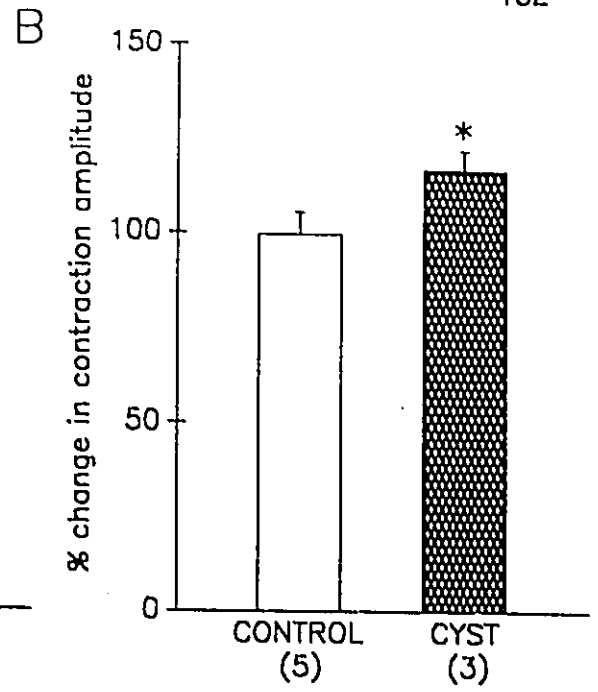
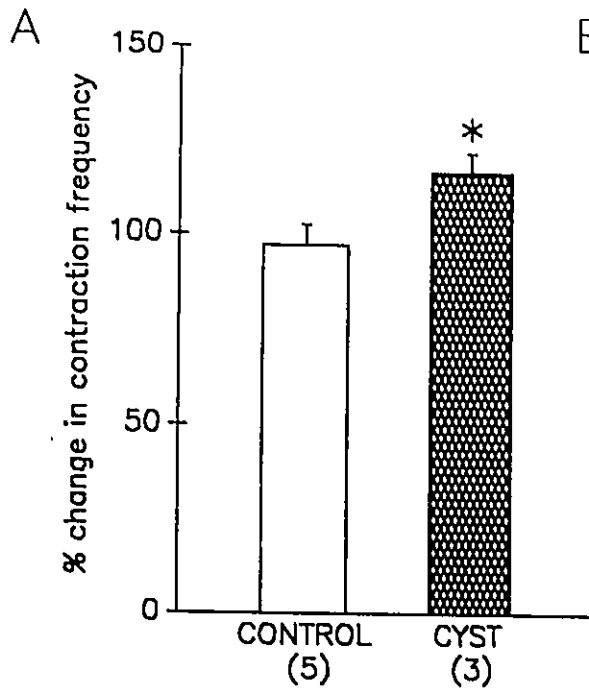


CYSTEAMINE



1 min

Figure 39. Comparison of the a) frequency; b) amplitude; c) duration and d) amount of duodenal contractile activity recorded at D2 (4cm distal to the pylorus) between control and cysteamine-HCl treated rats. The amount of contractile activity was determined from the area under the curve, and is presented as 'area'. Cysteamine-HCl (CYST) was given in 2 doses (28mg/100g sc). Data is presented in percentages, as the mean \pm SEM. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control group. The number (n) of rats in each treatment group is indicated in the abscissa.



was lower than those animals treated with cysteamine-HCl. Therefore, it is probable that cysteamine-HCl in fact caused no overall significant change in the contraction frequency of the duodenum.

In rats treated with cysteamine-HCl (n=3), a significant increase ($17 \pm 5\%$, $p < 0.05$) in contraction amplitude was measured compared to the control rats (n=5, Figure 39b). However, in two of three rats administered cysteamine-HCl, no significant increase ($p > 0.05$) in the amplitude of contraction was observed when compared to either the cysteamine-HCl control period or control rats.

Rats administered cysteamine-HCl (n=3) showed no significant change ($p > 0.05$) in contraction duration compared to the pre-cysteamine-HCl control period and control animals (n=5, Figure 39c).

The total amount of contractile activity, was not significantly affected ($p > 0.05$) following the administration of cysteamine-HCl (Figure 39d).

3.3.2d Summary.

The results from this study confirms earlier studies (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989) that cysteamine-HCl induces early alterations in normal gastroduodenal motility. Although in the present study not all rats responded to cysteamine-HCl treatment, this finding was not particularly surprising since it was previously observed (see Chapter 2) that the incidence of duodenal ulceration in the cysteamine-HCl rat model was

approximately 81%. Therefore, approximately 20% of all rats do not respond to cysteamine-HCl.

During the control recording period, contractile activity was the predominant motor activity observed in the rat gastroduodenum, although relaxations were also present. Cysteamine-HCl immediately disrupted normal, cyclical patterns of gastroduodenal motility, with these ulcerogen-induced effects persisting for up to six hours.

In the antrum, cysteamine-HCl significantly enhanced all measured parameters of contractile activity, resulting in an increased number of larger amplitude, longer duration contractions. However, in this same region, relaxations were rarely observed following treatment with cysteamine-HCl. On the basis of these results, it appears that cysteamine-HCl has stimulatory influences on contractile motor activity while exerting inhibitory effects on relaxant motor activity. The present work obtained from the rat antrum contradicts the findings of Pihan et al (1985) and Takeuchi et al (1987) who observed that cysteamine-HCl almost abolished gastric (corpus, midantrum) contractile activity.

Cysteamine-HCl has also previously been reported (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989) to rapidly (within 15 minutes) induce contractile hypermotility (lasting between three to six hours) of the proximal, mid and distal duodenum, which in many animals was followed by a more slowly developing (within 15 to 30 minutes), but long-lasting (up to 15 hours) period of hypomotility. In the present study, cysteamine-HCl also caused an

increase in contractile activity of the proximal duodenum. However, within the proximal duodenum, cysteamine-HCl was found to have region-specific actions. At D1, cysteamine-HCl significantly enhanced the frequency, amplitude and total area of contractile activity for up to six hours while at D2, the ulcerogen seldom induced any contractile motor changes. As observed in the antrum, cysteamine-HCl again exerted inhibitory influences on relaxant motor activity such that very few relaxations were observed.

The most probable reason for the discrepancy in the actions of cysteamine-HCl on gastrointestinal motility described herein, compared to previous studies, would be the recording technique employed. Previous recording techniques have the ability to measure smooth muscle contractile activity only but yet, motility consists of both contractions and relaxations for coordinated propulsion of intestinal contents. In fact, both contractions and relaxations of the intestine were visually observed and detected by the serosally positioned strain gauges, providing evidence that the motor activity recorded in these experiments were true motor events.

Previous in vitro studies using isolated rat stomach strips have shown that cysteamine-HCl induces concentration-dependent contractions predominantly through direct actions at muscarinic cholinergic receptor sites on the smooth muscle (Pihan et al, 1985; Krantis, 1987). Krantis (1987) also observed that these actions of cysteamine-HCl were selective, with contractions evoked in preparations of the rat forestomach and fundus but not antrum. However, Pihan et al (1985)

found that cysteamine-HCl induced contractile activity in all regions (forestomach, corpus, and antrum) of the rat stomach. In contrast to previous in vivo studies which demonstrated that cysteamine-HCl inhibited gastric motility (Pihan et al, 1985; Takeuchi et al, 1987), the present studies demonstrated a significant overall increase in antral contractile activity which correlated well to the in vitro studies. Although in the current work, the mechanism(s) involved in the cysteamine-HCl induced effects on contractile activity was not determined, the results obtained suggest two possibilities. Similar to in vitro studies, cysteamine-HCl may exert its actions directly on the smooth muscle. It is also possible that cysteamine-HCl may exert stimulatory actions on enteric, intrinsic cholinergic motor neurons since it has been previously demonstrated that this ulcerogen can have both myogenic and neural actions.

In contrast to its actions on isolated strips of the rat stomach, the predominant action of cysteamine-HCl on isolated segments of rat duodenum, was to induce concentration-dependent relaxations. These responses have been determined to be mediated by enteric, inhibitory non-adrenergic non-cholinergic (NANC) motor neurons, and involve cysteamine-HCl interactions with sites distinct from nicotinic and GABA receptors present to these neurons (Krantis, 1987; Krantis and Krause, 1989). Similar to previous in vivo studies, the current study also demonstrated that cysteamine-HCl induced rapid alterations in intestinal motility. However, in the present study, cysteamine-HCl was found to have region-specific actions, enhancing contractile activity at D1 but not at D2, while exerting

inhibitory influences on relaxant motor activity at both of these recording sites. The mechanism(s) of action and specificity of these effects induced by cysteamine-HCl are not known. However, it is unlikely that the predominant effect of this ulcerogen on contractile activity of the early proximal duodenum is through stimulation of intrinsic, inhibitory NANC motor nerves. In fact, it is possible that the distinct and region-specific actions of cysteamine-HCl along the proximal duodenum may help to localize the formation of duodenal ulcers to the first two centimeters of the duodenum.

The evidence obtained from the present studies suggests that alterations in gastroduodenal motility could interfere with normal neutralization and acid clearing mechanisms of the duodenal bulb, and in this manner, contribute to the development of duodenal ulceration.

3.3.3 Effects of GABA on Gastroduodenal Motility

Studies described in this thesis (see Chapter 2, section 2.1), and the work of others using the cysteamine-HCl rat model (for review see Chapter 1), have shown that peripherally administered γ -aminobutyric acid (GABA) aggravated cysteamine-HCl induced duodenal ulceration. This effect could be reversed in a concentration-dependent manner, by both the GABA_A-receptor antagonist bicuculline and the GABA_A-receptor coupled Cl⁻ channel blocker picrotoxinin. Taken together, these studies strongly suggest the involvement of peripheral

GABAergic sites in the development of duodenal ulcers. The presence of an extensive GABAergic system in the wall of the gastrointestinal tract (for review, see Chapter 2) suggests that this may be the target organ involved in these peripheral actions.

In vivo studies have provided evidence that the systemic administration of GABA alters the migrating myoelectric complexes of the rat intestine, with a primary period of inhibition followed by a phase of irregular spiking activity (Fargeas et al, 1988). This biphasic action of GABA is similar to the results obtained by Krantis et al (1980) using in vitro preparations of the guinea-pig intestine. More recently, cysteamine-HCl was demonstrated to reversibly reduce GABA-evoked NANC nerve-mediated relaxations in isolated segments of the rat small intestine, and these actions were unrelated to the GABA receptor sites on the inhibitory motor nerves (Krantis, 1987; Krantis and Krause, 1989).

The aim of these studies was to further investigate the extent of involvement of enteric GABAergic mechanisms in experimentally induced duodenal ulceration.

It has been previously shown (Chapter 2, section 2.1) that systemic GABA aggravates cysteamine-HCl induced duodenal ulcer and that cysteamine-HCl interacts with the same population of intrinsic enteric excitatory cholinergic motor neurons and inhibitory NANC neurons as GABA. It was then hypothesized that under the present in vivo conditions, that GABAergic treatment would modulate the effects of cysteamine-HCl on gastroduodenal motility.

The experimental protocol used in these studies was similar to Chapter 3, section 3.2. All rats received two subcutaneous injections of cysteamine-HCl, one hour apart, each at a dose of 28mg/100g and motility was recorded for up to 6 hours. Prior to the administration of cysteamine-HCl, rats were pretreated with γ -aminobutyric acid (GABA, 10mg/100g sc) plus amino-oxyacetic acid (AOAA, 2.5mg/100g sc to prevent GABA degradation). A three hour control recording was obtained from each rat before any drug treatment was initiated. The time-courses used for the various drug treatments are indicated in the figure legends.

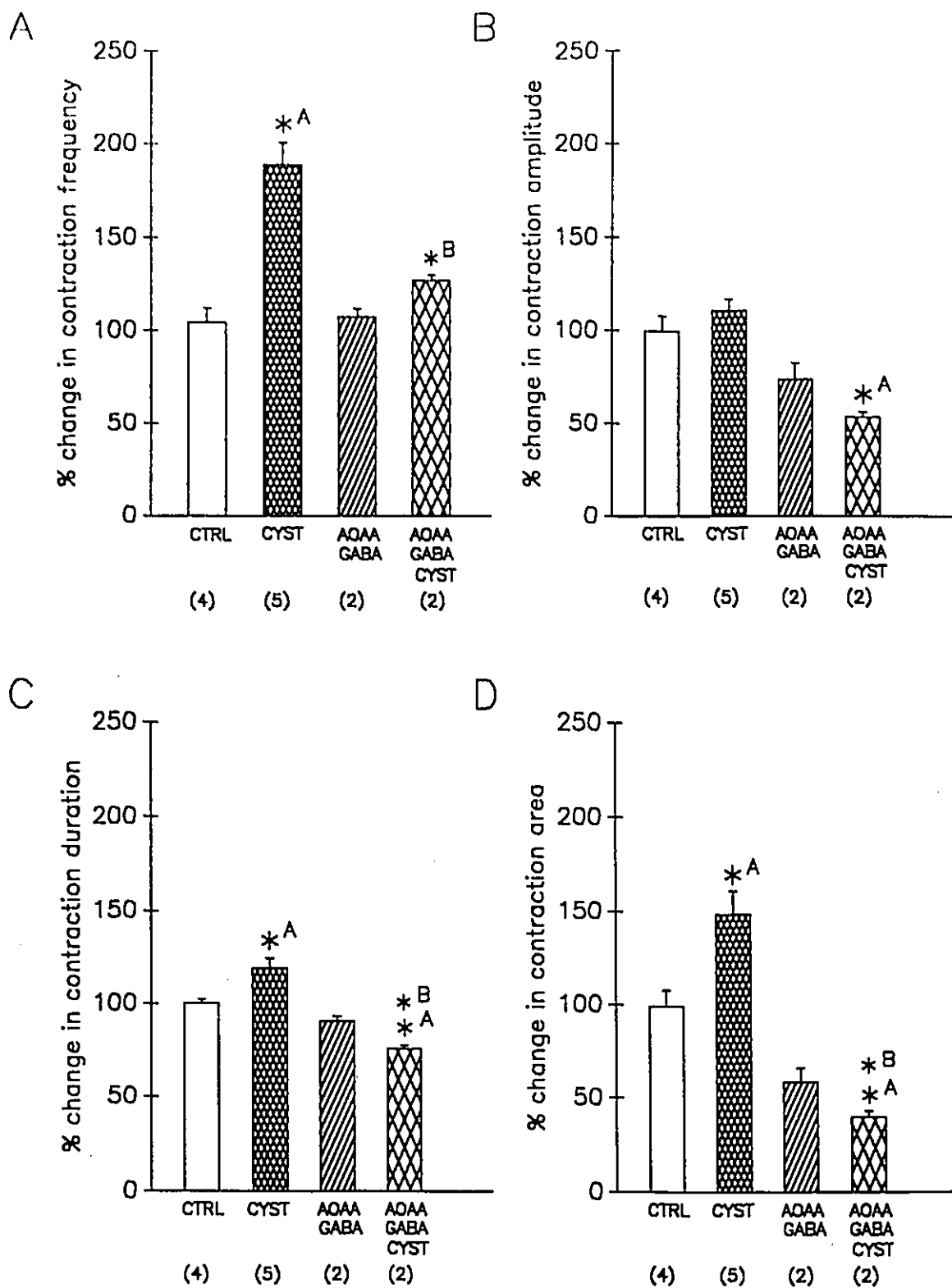
The effects of systemically administered GABA on contractile activity of the rat gastroduodenum are discussed below. Although both contractile and relaxant motor activity were recorded from these animals, the low frequency of relaxations did not permit valid statistical analysis.

3.3.3a Antrum

Antral Contractions

No significant changes ($p > 0.05$) in contraction frequency were observed in animals treated with AOAA alone ($n=2$), or with AOAA followed by GABA ($n=2$) compared to control rats ($n=4$). However, as shown in Figure 40a, the administration of GABA, 120 minutes following AOAA pretreatment, prevented ($p < 0.05$) the cysteamine-HCl-induced increase in contraction frequency. In fact,

Figure 40. Effects of GABA on rat antral contractions in cysteamine-HCl induced ulceration. a) frequency; b) amplitude; c) duration and d) amount of contractile activity. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Treatment groups are indicated in the abscissa. Cysteamine-HCl (CYST) was administered in 2 doses of 28mg/100g sc. GABA (10mg/100g sc) was given 90 minutes before cysteamine-HCl. AOAA (2.5mg/100g sc) was administered 210 minutes before cysteamine-HCl. Data is expressed in percentages, with the mean \pm SEM presented. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control (CTRL) group (**^A) or to the cysteamine-HCl (CYST) group (**^B). The number (n) of animals for the various treatment groups are indicated in the abscissa.



the frequency of contraction in rats pretreated with GABA (n=2) was not significantly different ($p>0.05$) from control animals (n=4).

The amplitude of contractions in animals treated with 1) cysteamine-HCl, 2) GABA plus AOAA, or 3) GABA plus AOAA plus cysteamine-HCl are illustrated in Figure 40b. One of two animals administered AOAA alone showed a reduction of $48 \pm 11\%$ in contraction amplitude compared to control rats (n=4). However, the amplitude of contractile responses recovered to control levels within 120 minutes following the AOAA injection. Treatment with GABA plus AOAA (n=2) had no significant effect ($p>0.05$) on the amplitude of contractions compared to the control group. However, in rats pretreated with GABA plus AOAA (n=2), the cysteamine-HCl (n=3) induced increase in contraction amplitude was prevented. Moreover, in these rats, the contraction amplitude was reduced ($47 \pm 3\%$, $p<0.05$) below that of the control group (n=4).

The duration of contractions was reduced ($22 \pm 3\%$) in one of two animals administered AOAA alone. However, within 120 minutes, this had recovered back to control level. Treatment with GABA plus AOAA (n=2) did not change ($p>0.05$) the duration of contractions compared to control animals (n=4), as summarized in Figure 40c. In rats pretreated with GABA plus AOAA (n=2), the cysteamine-HCl induced increase in contraction duration was prevented ($p<0.05$). In fact, contraction duration in these rats was significantly reduced ($24 \pm 2\%$, $p<0.05$) compared to control rats (n=4).

The total amount of contractile activity measured in 1) cysteamine-HCl, 2)

GABA plus AOAA, or 3) GABA plus AOAA plus cysteamine-HCl treated animals is presented in Figure 40d. Treatment with AOAA alone reduced ($68 \pm 7\%$, $p < 0.05$) the amount (area) of contractile activity in one of two rats, but recovery occurred within 120 minutes. Treatment of rats ($n=2$) with GABA plus AOAA did not affect ($p > 0.05$) antral contractility. However, GABA pretreatment ($n=2$) blocked ($p < 0.05$) the cysteamine-HCl induced increase ($n=3$) in contractile activity and in fact, reduced ($59 \pm 3\%$, $p < 0.05$) the total amount of contractile activity below that of control rats ($n=4$).

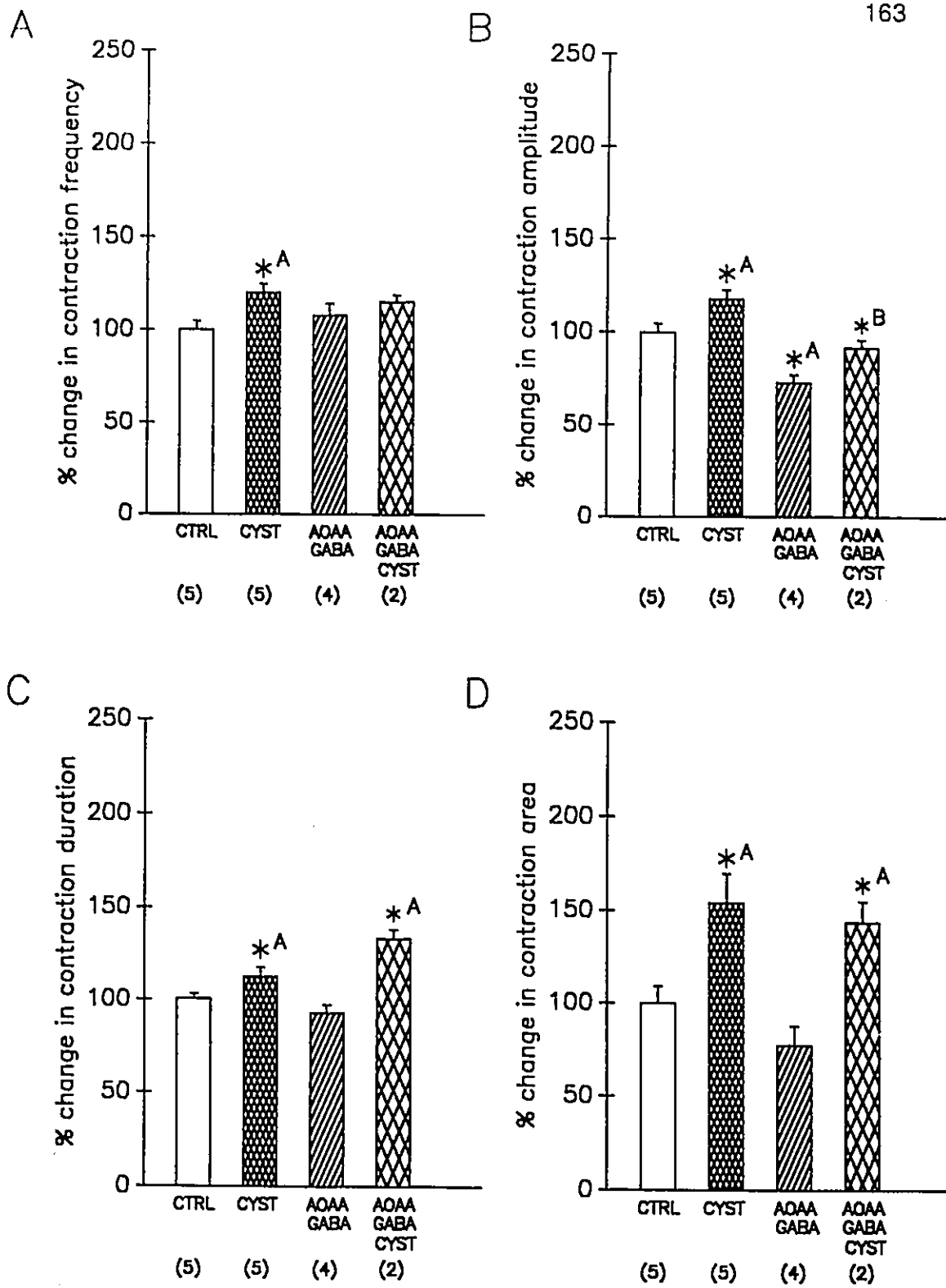
3.3.3b Duodenum - D1

Contractions at D1

Rats administered AOAA ($n=3$) alone demonstrated no significant changes ($p > 0.05$) in frequency, amplitude, duration or amount of contractions compared to control rats ($n=5$).

Treatment with GABA plus AOAA ($n=4$) caused no significant change ($p > 0.05$) in the contraction frequency compared to control animals ($n=5$), as presented in Figure 41a. Because of technical problems encountered during the recording sessions, the contraction responses of cysteamine-HCl treated rats pretreated with GABA plus AOAA could be statistically analyzed in only two of four rats. In these rats, GABA plus AOAA pretreatment did not prevent ($p > 0.05$) the

Figure 41. Effects of GABA on rat proximal duodenal (D1) contractions in cysteamine-HCl induced duodenal ulceration. a) frequency; b) amplitude; c) duration and d) amount of contractile activity. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Treatment groups are indicated in the abscissa. Cysteamine-HCl (CYST) was administered in 2 doses of 28mg/100g sc. GABA (10mg/100g sc) was given 90 minutes before cysteamine-HCl. AOAA (2.5mg/100g sc) was administered 210 minutes before cysteamine-HCl. Data is expressed in percentages, with the mean \pm SEM presented. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control (CTRL) group (*^A) or to the cysteamine-HCl (CYST) group (*^B). The number (n) of animals for the various treatment groups are indicated in the abscissa.



cysteamine-HCl-induced increase in contraction frequency.

As summarized in Figure 41b, GABA plus AOAA (n=4) significantly reduced ($26 \pm 4\%$, $p < 0.05$) the amplitude of duodenal contractions compared to control rats (n=5). However, these responses usually recovered to control levels within 90 minutes. Pretreatment with GABA plus AOAA (n=2) prevented ($p < 0.05$) the cysteamine-HCl (n=5) induced increase in contraction amplitude. In fact, the cysteamine-HCl treated rats pretreated with GABA plus AOAA demonstrated no significant change ($p > 0.05$) in contraction amplitude compared to control animals (n=5).

The contraction duration measured in rats treated with GABA plus AOAA (n=4) was not significantly different from control rats (n=5, Figure 41c). Cysteamine-HCl treated rats pretreated with GABA plus AOAA demonstrated no significant change ($p > 0.05$) in contraction duration compared to cysteamine-HCl treated animals alone (n=5), but a significant increase ($33 \pm 5\%$, $p < 0.05$) compared to control rats.

Treatment with GABA plus AOAA (n=4) reduced the total amount of contractile activity ($23 \pm 11\%$), but this decrease was not significantly different ($p > 0.05$) from control rats (n=5) as shown in Figure 41d. No significant change ($p > 0.05$) in the total amount of contractile activity was measured in cysteamine-HCl treated rats pretreated with GABA plus AOAA (n=2) compared to rats treated with the ulcerogen alone. However, the area of contraction in cysteamine-HCl treated rats pretreated with GABA plus AOAA demonstrated a significant increase ($44 \pm$

11%, $p < 0.05$) compared to the control group.

3.3.3c Duodenum - D2

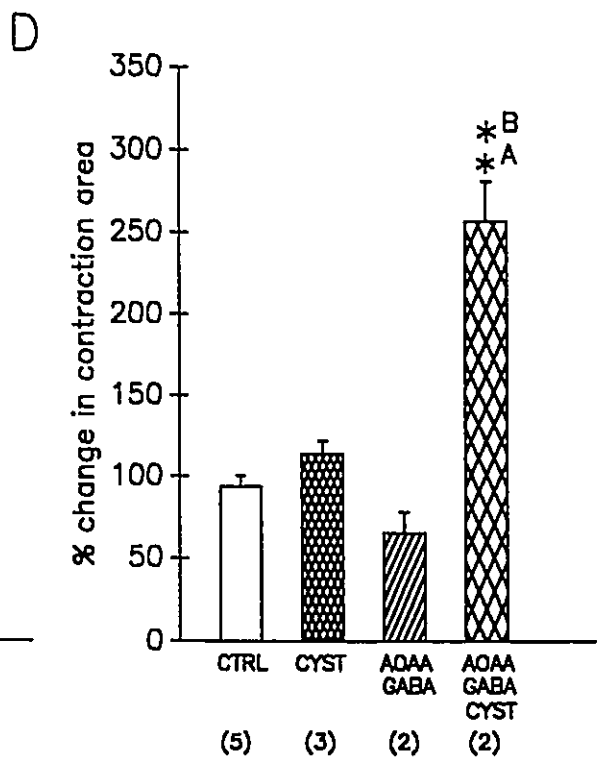
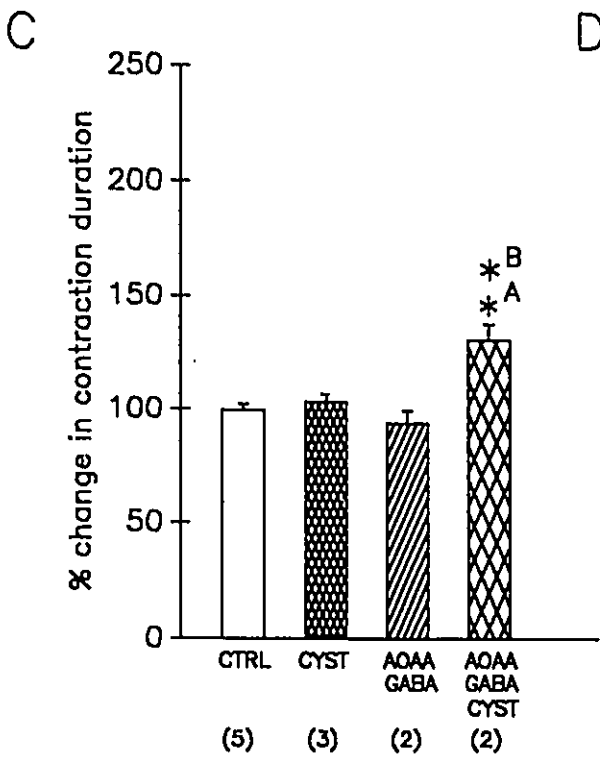
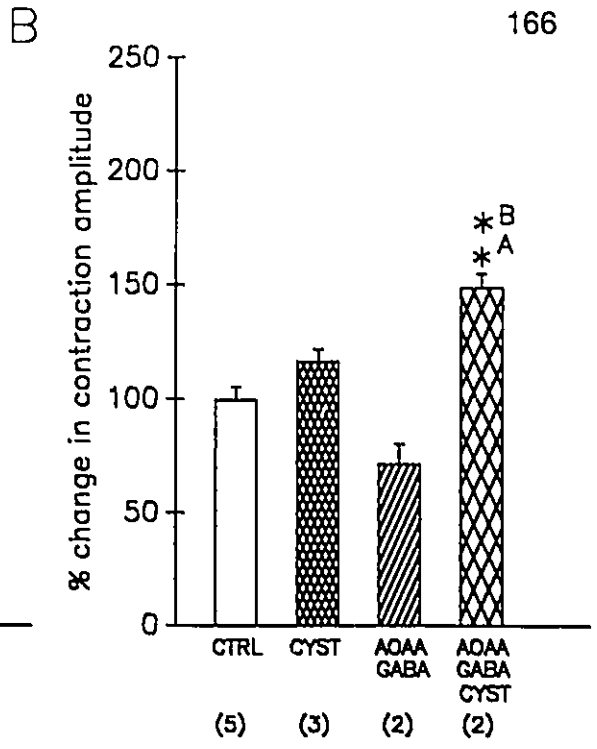
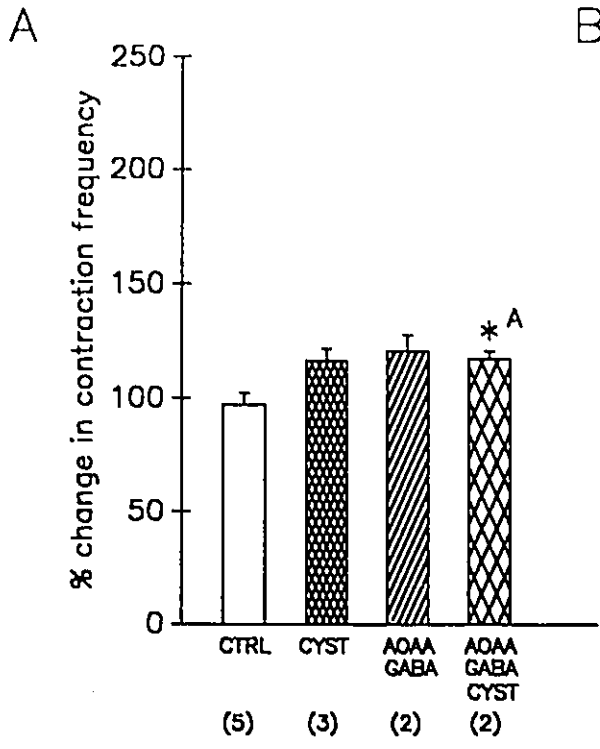
Contractions at D2

No significant changes ($p > 0.05$) in amplitude, duration or amount of contractile activity were observed in rats treated with AOAA alone ($n=2$). However, in these rats, contraction frequency was significantly increased ($25 \pm 6\%$, $p < 0.05$) compared to control rats ($n=5$). However, within 120 minutes, the frequency of contraction appeared to recover to control levels.

Treatment with GABA plus AOAA ($n=2$) increased the frequency of contraction compared to both AOAA-treated and control ($n=5$) animals, but this increase was not significant ($p > 0.05$, Figure 42a). Although cysteamine-HCl alone ($n=3$) induced no significant change in contraction frequency, animals pretreated with GABA plus AOAA ($n=2$) displayed a significant increase ($20 \pm 4\%$, $p < 0.05$) in frequency compared to control rats only.

GABA plus AOAA ($n=2$) reduced the contraction amplitude ($28 \pm 9\%$, but this decrease was not significantly different ($p > 0.05$) from control animals ($n=5$), Figure 42b. Cysteamine-HCl treated rats pretreated with GABA plus AOAA ($n=2$) demonstrated a significant increase in contraction amplitude compared to both cysteamine-HCl treated rats ($33 \pm 6\%$, $p < 0.05$) and control rats ($50 \pm 6\%$, $p < 0.05$).

Figure 42. Effects of GABA on rat duodenal (D2) contractions in cysteamine-HCl induced duodenal ulceration. a) frequency; b) amplitude; c) duration and d) amount of contractile activity. The amount of contractile activity was determined from the area under the curve and is presented as 'area'. Treatment groups are indicated in the abscissa. Cysteamine-HCl (CYST) was administered in 2 doses of 28mg/100g sc. GABA (10mg/100g sc) was given 90 minutes before cysteamine-HCl. AOAA (2.5mg/100g sc) was administered 210 minutes before cysteamine-HCl. Data is expressed in percentages, with the mean \pm SEM presented. Asterisks (*) denote a statistical significance of $p < 0.05$ compared to the control (CTRL) group (^{*A}) or to the cysteamine-HCl (CYST) group (^{*B}). The number (n) of animals for the various treatment groups are indicated in the abscissa.



The duration of duodenal contractions was not affected ($p>0.05$) by GABA plus AOAA treatment (Figure 42c). However, in cysteamine-HCl treated rats pretreated with GABA plus AOAA ($n=2$), a significant increase ($p<0.05$) in contraction duration was observed compared to both cysteamine-HCl treated ($n=3$) and control ($n=5$) rats ($27 \pm 7\%$ and $31 \pm 7\%$ respectively).

No significant change ($p>0.05$) in the amount of contractile activity was measured in GABA plus AOAA treated rats ($n=2$) compared to control rats ($n=5$). However, as summarized in Figure 42d, cysteamine-HCl treated rats pretreated with GABA plus AOAA demonstrated significant increases ($114 \pm 24\%$ and $162 \pm 24\%$; $p<0.05$) in contractile activity compared to both cysteamine-HCl treated ($n=3$) and control animals ($n=5$) respectively.

3.3.3d Summary.

In the present study, GABA, in the presence of AOAA, did not exert any significant overall effects on gastroduodenal motor activity. However, GABA often transiently diminished gastroduodenal motor activity and reduced larger amplitude contractions within five minutes of administration, with recovery of contractile activity occurring within 90 minutes. Since the present data analysis software calculates a single, average effect of a drug over the given time period, modifications to the program may be necessary to allow a more accurate investigation and description of shorter duration evoked disruptions to normal

motility patterns.

The effects of GABA on gastroduodenal motility observed in the present study correlates well with previous in vitro and in vivo studies. Fargeas et al (1988) showed that intraperitoneal administration of low doses (1 and 2mg/kg) of GABA in unanaesthetized rats was either ineffective or only transiently disrupted cyclical patterns of intestinal motility. At a higher dose (5mg/kg ip), GABA induced biphasic actions, with a primary phase of inhibition of spike burst activity followed by a period of irregular spiking activity. This dual action of GABA may relate to its neural action in the rodent intestine. Krantis et al (1980), using in vitro organ bath preparations of the guinea-pig intestine, demonstrated that GABA stimulated receptors on enteric cholinergic motor neurons and inhibitory non-adrenergic non-cholinergic NANC motor neurons. Recently, the rat has been shown to have a pharmacology similar to the guinea-pig (Krantis et al, 1980; Kerr and Ong, 1986; Maggi et al, 1986; Krantis and Harding, 1987). Thus, in the present study, the initial but transient influence of GABA in reducing contractile motor activity of the gastroduodenum may be mediated through intrinsic, inhibitory NANC motor neurons.

In the present work, a variety of responses were observed in cysteamine-HCl treated rats pretreated with GABA plus AOAA. In the rat antrum, pretreatment with GABA plus AOAA prevented the cysteamine-HCl induced increase in overall contractile activity, with the frequency, amplitude, duration and total amount of contractile activity reduced to or below the levels established in cysteamine-HCl

control rats. Under the experimental conditions of the present study, GABA, a known modulator of intestinal motility, would appear to reverse cysteamine-HCl induced gastric motor disturbances, potentially leading to a state of antral hypomotility.

In vitro studies of the rat stomach have previously demonstrated that the actions of cysteamine-HCl are mediated through intrinsic, excitatory cholinergic motor neurons, and by direct actions at muscarinic receptors in the muscularis (Pihan et al, 1985; Krantis, 1987; Krantis and Krause, 1989). Within the rat intestine, Krantis and coworkers (1987, 1989) found cysteamine-HCl to interfere with GABA stimulation of intrinsic, inhibitory NANC motor nerves. Thus, the ability of GABA to prevent cysteamine-HCl induced dysmotility of the rat antrum may possibly involve an interaction where the extent of stimulation of intrinsic cholinergic motor neurons by this ulcerogen is diminished. Unfortunately, the mechanism(s) involved in this apparent interaction between GABA and cysteamine-HCl is unknown.

In the proximal duodenum of cysteamine-HCl treated rats, pretreatment with GABA plus AOAA did not induce consistent effects on the frequency, amplitude, duration or total amount of contractile activity. In the early proximal duodenum (D1), pretreatment with GABA prevented cysteamine-HCl induced increases in contraction amplitude. However, in terms of frequency, duration and the total amount of contractile activity, GABA pretreatment did not change the cysteamine-HCl induced effects. At D2, pretreatment of cysteamine-HCl treated rats with

GABA increased the amplitude, duration and area of contractile motor activity. In addition, the frequency of contractions in these rats was increased compared to control animals. Thus, in contrast to cysteamine-HCl treatment alone, pretreatment with GABA appears to induce dysmotility over a greater length of the proximal duodenum. These observations may account for the previously observed (see Chapter 2) GABA-induced aggravation of cysteamine-HCl induced duodenal ulceration.

The results obtained from the present study also suggest that the effects of GABA on cysteamine-HCl induced alterations of gastroduodenal motility may be distinct, such that over relatively short distances, the antrum and proximal duodenum respond differently to the administration of GABA.

The mechanism(s) involved in the actions of GABA on cysteamine-HCl induced duodenal ulcers is presently unknown. However, some type of interaction appears to be occurring between GABA and cysteamine-HCl. Since Krantis (1987) and Krantis and Krause (1989) have shown that cysteamine-HCl interferes with the neural actions of GABA, then a likely site for this interaction would be through the intrinsic, cholinergic excitatory motor neurons.

CHAPTER 4

The results of this work show that manipulation of the peripheral GABAergic system can affect the development of experimental duodenal ulceration. Stimulation of peripheral GABA_A-receptor sites caused aggravation of cysteamine-HCl induced duodenal ulcer which could be reversed by systemically administered GABA antagonists. However, pharmacologic stimulation of GABA_B-receptor sites resulted in an amelioration of the experimental ulceration. Thus, depending on the receptor subpopulation, GABAergic treatment clearly modulated the extent, and severity of chemically-induced duodenal ulcers.

The mammalian GABAergic system is considerably more extensive than previously believed, with GABA functioning both as a neurotransmitter and neuromodulator of intrinsic gastrointestinal nerves. GABA, the major inhibitory neurotransmitter of the central nervous system (Meldrum, 1982) is now established as a transmitter of enteric interneurons in the guinea-pig, rat, cat and human (Krantis, 1982; Jessen et al, 1983; Erdö, 1985; Tanaka, 1985; Erdö and Kiss, 1986; Krantis and Harding, 1986; Jessen et al, 1987; Taniyama et al, 1987).

In addition, there is accumulating evidence for GABA to be localized in antral mucosal endocrine glandular cells where it exerts modulatory interactions on the release of gastrin and somatostatin (Harty et al, 1983, 1986; Jessen et al, 1988; Erdö et al, 1989; Guo et al, 1989; Erdö et al, 1990; Gilon et al, 1990; Krantis

and Clark, 1991). GABA_A-receptors are present on circular smooth muscle, gastric mucosal and small intestinal gland cells and intestinal enterochromaffin cells (Jessen et al, 1988; Erdö et al, 1989; Schwörer et al, 1989; Erdö et al, 1990; Gilon et al, 1990; Harty et al, 1991). Furthermore, GABA and its enzymes of biosynthesis have been identified in both β cells and a subpopulation of somatostatin D cells in the islets of Langerhans of the pancreas (Vincent et al, 1983; Okada, 1986; Gilon and Remacle, 1989). Thus, it is now evident that the enteric GABAergic system is both large and extensive, with the potential to play an important role in both motility and secretomotor functions of the gut.

It is now apparent that there is a triad of factors associated with the development of duodenal ulcers, including aggressive factors such as acid secretion, reduced duodenal defense mechanisms and altered gastroduodenal motility. The acid load presented to the duodenal bulb overcomes the normal neutralizing capability of the mucosa, to result in ulcer formation. The size of the acid load is dependent on two factors, acid secretion and the rate at which the acid is emptied into and removed from the duodenum, thus implicating motor activity of the gastroduodenum. Moreover, the findings of the present study demonstrate the involvement of GABA in duodenal ulcer, which supports the initial proposal of Szabo (1984) that the neuroendocrine status of the animal does indeed appear to be important in this disorder.

Structure-activity studies have provided evidence that a resemblance exists between duodenal ulcerogens and certain neurotransmitters, including GABA,

histamine, dopamine and acetylcholine (Szabo et al, 1982). On the basis of these types of studies, certain endogenous substances, including GABA, have been investigated and shown to exert modulating actions on the induced ulceration. However, the nature of this modulation is unclear.

The development of experimental duodenal ulcers has been shown to be accompanied by biochemical changes in certain neurotransmitter systems, such as the catecholamines, 5-hydroxytryptamine (5-HT), histamine and γ -aminobutyric acid (GABA) in both central and/or peripheral tissues. However, these alterations in neurotransmitter systems are only one of a number of changes or disruptions that occur during the development of cysteamine-HCl induced duodenal ulceration.

The results obtained from the present study confirmed and extended the preliminary findings of Krantis and Nicholson (1989), with peripherally administered GABA agonists and antagonists pharmacologically manipulating cysteamine-HCl induced duodenal ulceration. Specifically, pretreatment with GABA increased the incidence and severity of cysteamine-HCl induced duodenal ulcers, with the majority of rats showing evidence of opposing lesion formation. It has previously been shown that systemically administered GABA cannot readily cross the blood-brain-barrier (Oldendorf, 1971; Rapoport, 1976). In addition, each rat pretreated with GABA received a single bolus injection of amino-oxyacetic acid (AOAA) to prevent the degradation of GABA, resulting in increased GABA levels in the periphery only. Thus, the modulatory actions of GABA on experimental ulcer in these studies would appear to be mediated through peripheral GABA receptors.

The GABA aggravation of cysteamine-HCl induced ulceration could be reversed in a concentration-dependent manner by the specific GABA_A-receptor antagonist bicuculline and the GABA-associated Cl⁻ ionophore channel blocker picrotoxinin. Both GABA antagonists were equally effective in reducing the incidence, number of ulcers (ie. ulcer profile) and severity of ulceration. In fact, these antagonists of GABA appeared to exert protective, anti-duodenal ulcer actions, since the resulting ulceration was less severe than that observed in cysteamine-HCl treated control rats. The effects of GABA and GABA antagonists on cysteamine-HCl induced duodenal ulcer has been schematically presented in Figure 43.

Although bicuculline and picrotoxinin reduced each of the measured parameters of ulceration to a similar extent, the most dramatic effects of these antagonists was the reduction in lesion area and the shift in ulcer profile, with approximately half of the rats being completely free of duodenal ulcers.

Bicuculline and picrotoxinin are both able to penetrate the blood-brain-barrier and thus, the anti-duodenal ulcer actions of these antagonists may involve interactions with central GABAergic receptor sites. Szabo et al (1982) provided evidence that centrally (icv) administered GABA increased the severity of cysteamine-HCl induced duodenal ulceration while GABA antagonists were found to exert protective actions. On this basis, Szabo et al (1982) proposed that central, but not peripheral GABAergic sites are important to the manipulation of the induced ulceration. However, the present study, in agreement with Krantis and

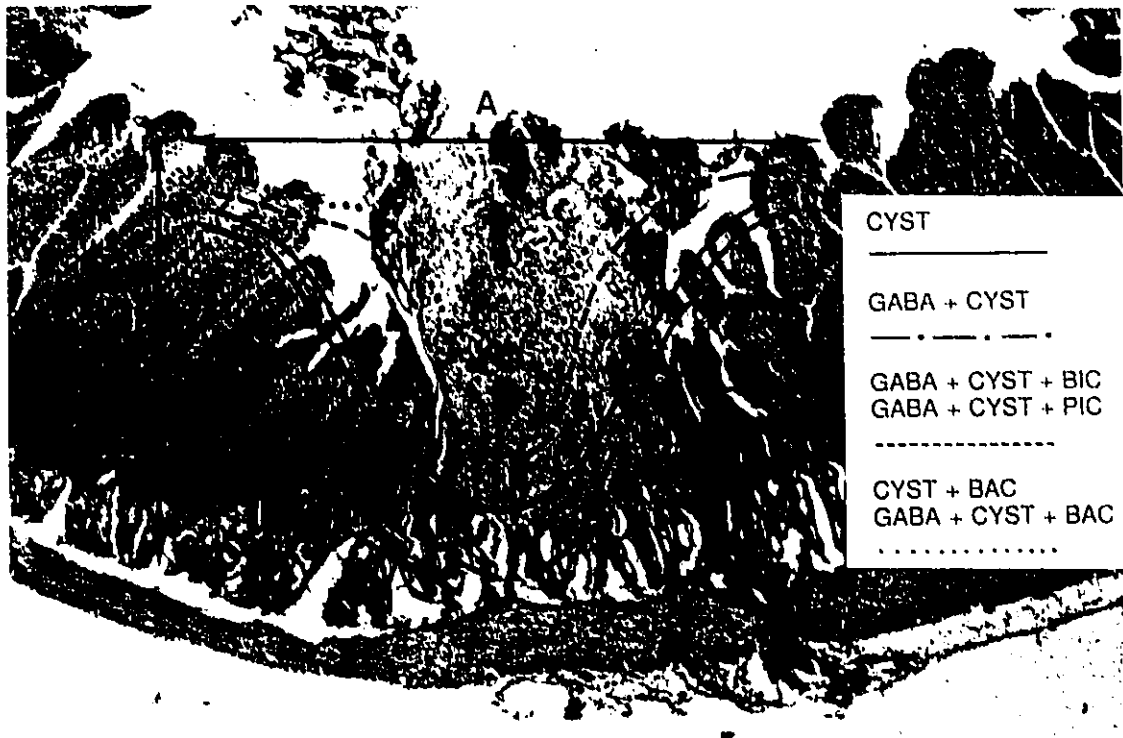


Figure 43. Schematic diagram illustrating the effects of various GABAergic treatments on the severity of cysteamine-HCl induced duodenal ulcer. Treatments are indicated alongside. The various lines are superimposed over a paraffin section of a typical duodenal ulcer in the rat, showing the relative effects of these treatments on lesion area (A) and transmural intensity (I). CYST = cysteamine-HCl. GABA = γ -aminobutyric acid. BIC = bicuculline. PIC = picrotoxinin. BAC = baclofen.

Nicholson (1989), provides convincing evidence for a role of peripherally located GABAergic sites in cysteamine-HCl induced duodenal ulcer. Indeed, enteric neural mechanisms may be important factors underlying the development of duodenal ulcers. Therefore, it would appear that both central and peripherally located GABAergic sites play an important role in the modulation of duodenal ulcer disease.

The mechanism(s) involved in the observed aggravation of cysteamine-HCl induced ulceration by GABA has not been determined. However, the actions of GABA and GABA antagonists on experimental ulceration may be related to enteric GABA_A-receptor stimulation of gastroduodenal motility and/or acid secretion.

Previous studies have shown that GABA and GABA_A-related analogues (muscimol) can modulate gastric acid secretion through central and peripheral GABA_A-receptor sites. Some studies demonstrate that GABA and GABA_A-specific analogues stimulate gastric acid secretion (Levine et al, 1981; Szabo et al, 1982; Harty et al, 1983, 1986; Tsai et al, 1987; Blandizzi et al, 1988; Guo et al, 1989; Del Tacca et al, 1990). However, other studies provide evidence that GABA inhibits gastric acid secretion and stimulates gastric mucous secretions, exerting mucocytprotective effects on the gastric mucosa (Bhargava et al, 1985; Lloyd et al, 1986; Minano et al, 1987; Erdö et al, 1989).

In the present study, the increased incidence and severity of duodenal ulcers by systemically administered GABA may be the result of GABA-induced acid secretion through the activation of antral GABA_A-receptors. Moreover, cysteamine-

HCl treatment alone has previously been shown to increase acid secretion (Groves et al, 1974; Ishii et al, 1976; Szabo et al, 1977; Tamaki et al, 1978; Kirkegaard et al, 1980; Tanaka et al, 1986) and diminish duodenal HCO_3^- secretion (Kirkegaard et al, 1981; Adler et al, 1983; Bridén et al, 1985; Ohe et al, 1988). Thus, GABA aggravation of cysteamine-HCl induced duodenal ulceration may involve further enhanced stimulation of gastric acid secretion, such that an even larger acid load is presented to the proximal duodenum, overcoming the already reduced duodenal defense mechanisms thus, predisposing this region to ulcer formation. In addition, GABA_A antagonists have been shown to reverse the GABA-induced effects on acid secretion (Tsai et al, 1987), suggesting that the anti-duodenal ulcer actions of these agents may be mediated through GABA_A -receptor sites. However, since duodenal ulcer is a multi-factorial disease, it is highly unlikely that the effects of GABA on acid secretion alone can completely account for the augmenting effects of this transmitter on cysteamine-HCl induced duodenal ulceration.

The earliest described disruption associated with cysteamine-HCl induced duodenal ulcer is altered gastroduodenal motility, occurring within five to 15 minutes following treatment with the ulcerogen (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). On the basis of the evidence obtained from both *in vivo* and *in vitro* studies, motility would appear to influence experimental ulcer. It appears likely that the modulatory effects of GABA and GABA_A antagonists on the ulcerogenic effects of cysteamine-HCl are due to the effects of the GABAergic agents on secretory and/or motor functions of the

gastroduodenum.

In contrast to the effects of stimulation of peripheral GABA_A-receptors, stimulation of GABA_B-receptors by baclofen (Lioresal), resulted in an amelioration of both cysteamine-HCl and GABA-aggravated cysteamine-HCl induced duodenal ulceration. These results provide further support for the involvement of peripheral GABAergic mechanisms in experimental ulcer.

Baclofen effectively reduced all measured parameters of cysteamine-HCl and GABA-aggravated ulceration. However, the magnitude of reduction for incidence, ulcer profile (0, 1 or 2 ulcers) and severity of ulceration was not the same. The greatest effects of baclofen were observed on the lesion area and ulcer profile which was similar to that observed with the GABA antagonists bicuculline and picrotoxinin. In fact, at least half of the baclofen pretreated rats showed no evidence of ulceration.

Similar to the actions of the GABA antagonists bicuculline and picrotoxinin, baclofen not only reversed the GABA aggravation, but reduced the extent and severity of ulceration below that of cysteamine-HCl treated control rats. In terms of the severity of ulceration, both cysteamine-HCl and GABA-aggravated cysteamine-HCl induced ulceration were reduced to the same level by baclofen pretreatment. Baclofen was able to counteract and reduce the effects of GABA. Thus, baclofen, in addition to possessing anti-spastic properties (Olpe et al, 1978), appears to exert powerful, anti-duodenal ulcer actions. The dose of baclofen used in the present study was identical to that used by Fargeas et al (1988) who found

that the effects of intraperitoneal (ip) administration of baclofen could be reproduced centrally (icv) at a dose which was a thousand-fold lower. Fargeas et al (1988) also demonstrated that the effects of baclofen (ip) could be prevented by the cholinergic muscarinic antagonist atropine (ip or icv). However, current evidence has shown that baclofen only poorly penetrates the blood-brain-barrier (Bowery and Pratt, 1992; Bowery, 1993). Therefore, it is likely that the actions of this highly lipophilic compound (Faigle and Keberle, 1972), baclofen, are mediated through mainly peripheral GABA_B-receptor sites.

Although both bicuculline and picrotoxinin were effective in reversing the GABA aggravation of cysteamine-HCl induced effects, baclofen appeared to be slightly more powerful, causing greater reductions in the ulcer profile and transmural depth (intensity) of the ulceration. These actions of baclofen are schematically illustrated in Figure 43.

In the present study, it is highly unlikely that baclofen stimulated acid secretion since this GABA_B-receptor ligand demonstrated powerful, anti-duodenal ulcer actions. Indeed, the dose of baclofen used in this study was 10 to 1000 fold greater than that used in studies where this agent was found to inhibit gastric acid secretion (Lloyd et al, 1986; Del Tacca et al, 1990). Thus, the ameliorating actions of baclofen on both cysteamine-HCl and GABA-aggravated cysteamine-HCl induced duodenal ulceration may be mediated, in part, through inhibitory influences on gastric acid secretion. Indeed, protective, anti-duodenal ulcer actions were also exerted by the GABA antagonists, bicuculline and picrotoxinin, and these agents

have been previously demonstrated to inhibit acid secretion.

In addition to its apparent effects on acid secretion, baclofen also has modulatory actions on gastrointestinal motility. Although the in vivo actions of baclofen on gastrointestinal motor activity contradict in vitro studies (Ong and Kerr, 1983; Giotti et al, 1983; Andrews and Wood, 1986; Krantis and Harding, 1987; Fargeas et al, 1988), it is clear that baclofen does exert influences on gastrointestinal motility. Therefore, the protective actions of baclofen on experimental ulceration may be due not only to an inhibition of acid secretion, but also to an effect on gastroduodenal motility.

If baclofen is influencing intestinal motility to reduce chemically-induced duodenal ulcer, it is unlikely that this agent is exerting stimulatory effects as observed in previous in vivo studies. Increased contractile motor activity has been observed following cysteamine-HCl treatment which is eventually followed by lesion development. However, baclofen pretreated rats show significant reductions in experimental ulceration. It is possible, as demonstrated from in vitro studies, that baclofen may diminish gastrointestinal motility, and thus, correct cysteamine-HCl evoked motor dysfunctions to protect the mucosa against the induced ulceration.

GABA antagonists, such as bicuculline and picrotoxinin, may have similar mechanisms of actions to that of baclofen in protecting against experimental ulceration, since these agents appear to exert modulating influences on both motor function and secretory processes within the gastroduodenum.

The primary pharmacological therapy for duodenal ulcer disease is focused

on either inhibition or neutralization of acid secretion, with the current standard treatment being the use of the histamine H₂-receptor blocker cimetidine. Within the literature, the effects of cimetidine on cysteamine-HCl treated rats is controversial, with some studies reporting significant reductions in duodenal ulceration (Okabe et al, 1977; Szabo et al, 1979; Poulsen et al, 1986; Tanaka et al, 1989), while other studies demonstrate no apparent effect (Stiel et al, 1983; Narasaki et al, 1985).

In the present work, the apparent protective, anti-duodenal ulcer actions of baclofen were compared to the actions of the H₂-receptor antagonist cimetidine on both cysteamine-HCl and GABA-aggravated cysteamine-HCl induced duodenal ulceration. Cimetidine, was observed to be less effective than baclofen in reducing cysteamine-HCl induced duodenal ulcer. In addition, cimetidine was completely ineffective in preventing the GABA aggravation of experimental ulceration. Although cimetidine reduced the incidence and transmural depth of cysteamine-HCl induced ulceration, half of the treated rats showed evidence of opposing lesion formation. The majority of baclofen pretreated rats were free of ulcers and all measured parameters of ulceration were reduced. Furthermore, cimetidine treatment did not protect against GABA-induced aggravation whereas baclofen again exerted powerful, anti-duodenal ulcer actions.

Cimetidine exerts protective effects on experimental ulceration through inhibition of gastric acid secretion. In cysteamine-HCl treated rats, cimetidine caused a small reduction in the induced ulceration. However, in cysteamine-HCl

induced duodenal ulcers aggravated by GABA, cimetidine treatment did not diminish any of the measured parameters of ulceration, suggesting that some factor(s), other than acid secretion, must be involved in the ulcerogenic process.

Cimetidine has been found to exert little effect on motility (Okabe et al, 1977) whereas GABA, GABA antagonists and baclofen, are all involved in the modulation of gastrointestinal motility. This is an important observation because it further implicates a role for motility in the pathogenesis of duodenal ulceration. Motility alterations precede functional, secretory, biochemical and morphological changes of the proximal duodenum in cysteamine-HCl treated rats (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). These studies, taken together with the current work, suggest that correction of gastrointestinal motor disturbances may represent a potentially new therapy for the treatment of this disease.

GABA, GABA antagonists and baclofen potentially affect gastric acid secretion and motility, two of the three known factors of ulceration. Thus, the more powerful, anti-duodenal ulcer actions of GABA antagonists and baclofen in comparison to cimetidine, may be a direct consequence of the number of factors affected in the triad of ulcer-inducing factors.

The present study provides compelling evidence that enteric GABAergic sites are important to the modulation of cysteamine-HCl induced duodenal ulceration. These findings should allow further insight into the role of the GABAergic system to the overall enteric nervous control of gastrointestinal

function. Moreover, the discovery that when stimulated, the two receptor populations (GABA_A and GABA_B) for GABA exerted opposite effects on experimental ulceration, suggests that these receptor sites may become important therapeutic target sites for the treatment of duodenal ulcer disease.

These kinds of studies provide new and important data for improving treatment of this disease, since there is still no cure. The pharmacological treatments employed for these patients do not have a high success rate. In duodenal ulcer patients, the relapse rate of duodenal ulcers following cessation of cimetidine therapy can reach as high as 80% (Korman et al, 1980; Ippoliti et al, 1983; Lane and Lee, 1988; Pan and Liao, 1990; Pan et al, 1991). Clearly, more appropriate therapies are required for the treatment of this disease. Thus, many laboratories are currently investigating new pharmacological regimens for treating duodenal ulcers. In addition to the study of the involvement of the GABAergic system in the development of duodenal ulceration, I also tested a new drug, U74500A, which belongs to a group of compounds called the 'lazaroids' developed by the Upjohn Company. Previous studies have shown these compounds to be potent inhibitors of lipid peroxidation (Braugher et al, 1989; Braugher and Pregoner, 1989; Hall and Pazara, 1989) and possess iron-chelating properties (Braugher et al, 1988, 1989). In fact, U74500A has been used in the treatment of renal and bowel ischemia.

U74500A (21-(4-(3,6-bis(diethylamino)-2-pyridinyl)-1-piperazinyl)-16 α -methyl-pregna-1,4,9(11)triene-3,20-dione, hydrochloride) has previously been

demonstrated to be ineffective in protecting against ethanol-induced gastric damage (Krantis et al, 1992). However, in the present study, this compound exerted powerful, anti-duodenal ulcer actions on cysteamine-HCl and GABA-aggravated cysteamine-HCl induced duodenal ulceration. U74500A effectively reduced all measured parameters of ulceration. In fact, this lazaroid appears to possess anti-duodenal ulcer actions since it reduced the extent and severity of both cysteamine-HCl and GABA-aggravated duodenal ulcers below the established control levels.

U74500A did not cause a similar magnitude of reduction for each of the measured parameters of ulceration. The most dramatic effects of this drug were in the profile of ulceration (0, 1 or 2 ulcers) and the area of mucosal damage.

When the anti-duodenal ulcer actions of U74500A were compared to that of baclofen, and the GABA antagonists bicuculline and picrotoxinin, the reduction in the induced ulceration was similar. However, slightly more baclofen pretreated rats were completely free of duodenal lesions. In addition, U74500A was considerably more effective than cimetidine in protecting against the experimentally-induced duodenal ulcers. Although the mechanism(s) of this anti-ulcer action of U74500A is presently unknown, based on the powerful actions of this compound on duodenal ulceration, it is probable that like the GABA antagonists and baclofen, more than one factor within the so-called triad of ulcer factors is being affected. This notion is supported by the initial findings obtained from in vitro preparations of isolated rat proximal duodenum and distal jejunum,

where U74500A induced concentration-dependent contractions and was observed to interact/interfere with both cholinergic muscarinic and serotonergic mechanisms.

In these studies, U74500A did not affect the ability of the intestine to relax to the nicotinic, cholinergic agonist DMPP; to electrical stimulation of the intrinsic, NANC inhibitory motor nerves or to histamine-induced relaxations. However, contractions induced by the muscarinic agonist carbachol and 5-HT-induced contractions were reduced in the presence of this lazaroid. Thus, the actions of U74500A appeared to be specific, diminishing contractile motor responses only. On the basis of this evidence, U74500A may exert its protective actions on the development of duodenal lesions by exerting inhibitory influences on cysteamine-HCl enhanced contractile activity of the duodenum.

Although the direct effect of U74500A on gastric acid secretion has not yet been investigated, the apparent anti-muscarinic actions of U74500A may also partially explain its powerful, anti-duodenal ulcer actions, since it has been previously shown that anti-muscarinic agents effectively reduce gastric acid secretions (Kromer and Gönne, 1988).

It appears possible that U74500A, similar to the GABA antagonists and baclofen, may exert influences on both gastrointestinal motor function and secretory processes.

The pharmacological studies employing the cysteamine-HCl rat model described herein, provide further support to the proposal that motility, in addition to other factors including acid secretion, may be responsible for the observed

modulation of experimental ulceration, and may represent a new avenue for the treatment of this disease.

To investigate the possible role of motility in experimental duodenal ulceration, in vivo motility recordings of the gastroduodenum were obtained, using a newly developed method of serosally placed miniaturised extraluminal strain gauges. This method allowed, for the first time, accurate recording of both contractions *and* relaxations, important components for normal peristalsis of the gastroduodenum.

The results obtained from the present study are in agreement with previous studies (Szabo et al, 1985; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989) that cysteamine-HCl rapidly induces alterations in normal gastroduodenal motility. However, in the present work, cysteamine-HCl caused immediate disruptions to normal motility whereas other studies report a delay of five to 15 minutes.

In the rat antrum, cysteamine-HCl increased contractile motor activity, such that large amplitude, longer duration contractions were observed while exerting an apparent inhibitory influence on relaxant motor activity. Indeed, relaxations were only occasionally observed following cysteamine-HCl treatment. These results contradict previous in vivo findings of Pihan et al (1985) and Takeuchi et al (1987) who both reported cysteamine-HCl to almost completely abolish gastric (corpus, midantrum) contractile motor activity. However, in vitro studies, using isolated preparations of the forestomach, corpus, antrum or fundus, have demonstrated

cysteamine-HCl to induce concentration-dependent contractions predominantly through direct actions at muscarinic receptors on the smooth muscle (Pihan et al, 1985; Krantis, 1987; Krantis and Krause, 1989). On the basis of this evidence, the observed actions of cysteamine-HCl on antral contractile activity in the present study may be due to its actions on the smooth muscle. However, since cysteamine-HCl has also been shown to have neural actions in the intestine, exerting its effects through stimulatory sites on intrinsic, inhibitory NANC motor neurons; inhibitory sites on excitatory cholinergic motor neurons and through inhibitory sites on intestinal NANC neurons, the possibility also exists that these neurally-mediated actions may be responsible for the cysteamine-HCl induced effects on antral motor activity. Thus, disruption or antagonism of intrinsic NANC inhibitory motor neurons by cysteamine-HCl may explain the loss of antral relaxation motor activity following treatment with this ulcerogen.

In the present study, cysteamine-HCl induced regional specific actions on duodenal motility, increasing contractile activity at D1 but not at D2 while diminishing relaxant motor activity (D1 and D2) for up to six hours. This apparent specificity in the actions of cysteamine-HCl on duodenal motility may account for the subsequent development of duodenal ulcers within the first 2cm distal to the pylorus. Previous in vivo studies have not reported any regional specificity in the actions of this ulcerogen. In fact, these studies report cysteamine-HCl to induce contractile hypermotility of the proximal, mid and distal duodenum (lasting between three and six hours), which in many animals was followed by a long-lasting period

(up to 15 hours) of hypomotility (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989). In vitro studies provide evidence that does not correlate to in vivo studies. From in vitro studies using isolated segments of the rat duodenum, the primary action of cysteamine-HCl was to evoke concentration-dependent relaxations, mediated through stimulation of enteric, inhibitory NANC motor nerves, at sites distinct from nicotinic and GABA_A-receptors (Krantis, 1987; Krantis and Krause, 1989).

The mechanism(s) and specificity of cysteamine-HCl induced effects on duodenal motility in vivo is currently unknown. Within the proximal duodenum, it is unlikely that the predominant action of cysteamine-HCl is to stimulate the inhibitory NANC nerves. However, since cysteamine-HCl also exerts its effects through inhibitory sites on enteric NANC motor nerves, this may explain the inability of the intestine to relax following administration of the ulcerogen. Thus, under the in vivo conditions of the present study, it is likely that inhibition predominated over stimulation of the NANC motor neurons. Since cysteamine-HCl has been established to have myogenic and neural mechanisms of action, it is also possible that the induced proximal duodenal hypermotility may be mediated via stimulation of the intrinsic, excitatory cholinergic motor neurons or through direct actions on the smooth muscle.

The results of this work, taken together with previous in vivo and in vitro motility studies (Bakich et al, 1984; Szabo et al, 1984; Pihan et al, 1985; Krantis, 1987; Takeuchi et al, 1987; Krantis and Krause, 1989; Mangla et al, 1989) provide

convincing evidence that alterations in normal gastroduodenal motility, in addition to secretory disorders, are important to the pathogenesis of cysteamine-HCl induced duodenal ulceration. In fact, altered motility may be one of the initial precipitating factors of duodenal ulcer disease. Thus, abnormal motility of the gastric antrum potentially interferes with gastric emptying, thereby affecting the acid load in the proximal duodenum. Cysteamine-HCl induced duodenal ulceration is also associated with a reduced duodenal mucosal resistance. Altered duodenal motility has been proposed to interfere with diffusion of pancreatic and biliary HCO_3^- from more distal regions of the duodenum, such that a large portion of the acid in the duodenal bulb is not neutralized, and overcomes the weakened resistance of the duodenal mucosa to result in ulcer formation (Pihan et al, 1985). Therefore, the observed contractile hypermotility of the duodenum, in the presence of reduced relaxant motor activity described in this study, may contribute to the pathogenesis of duodenal ulcer disease by interfering with normal acid clearing and neutralization mechanisms, leading to reduced pH within the proximal duodenum and subsequent development of duodenal lesions.

The cysteamine-HCl induced alterations in gastroduodenal motility observed in the present study, as well as in other studies (Szabo et al, 1984; Pihan et al, 1985; Takeuchi et al, 1987; Mangla et al, 1989) provides further evidence that correction of motor dysfunctions may be beneficial in preventing the development of duodenal ulcers. In fact, the protective, anti-duodenal ulcer actions of the GABA antagonists bicuculline and picrotoxinin; the GABA_B-receptor ligand baclofen and

the 21-aminosteroid U74500A, all potentially involved effects on gastrointestinal motor function. Thus, the in vivo effects of each of these agents on cysteamine-HCl induced disruptions of gastroduodenal motility needs to be investigated.

Krantis and coworkers (1987, 1989) have shown that in the rat intestine in vitro, cysteamine-HCl interferes with GABA stimulation of intrinsic, inhibitory NANC motor nerves. From the results of the present in vivo studies, GABA appears to prevent (antrum) and augment (duodenum) cysteamine-HCl-induced effects on gastroduodenal motility. This suggests that some type of interaction is occurring between these agents. The extent to which these GABA-induced effects on motility are related to the aggravating actions of GABA on cysteamine-HCl induced duodenal ulcer remains to be determined.

4.1 Conclusion.

The results obtained from in vivo pharmacological and physiological studies presented in this thesis, provide support for a role of the peripheral GABAergic system in the development of experimental duodenal ulcers. Stimulation of peripheral GABA_A-receptors augmented cysteamine-HCl induced duodenal ulceration which could be reversed by GABA antagonists. Stimulation of GABA_B-receptors by baclofen (Lioresal) was found to ameliorate experimental duodenal ulceration.

Evidence obtained from the in vivo motility recordings from conscious, unrestrained rats demonstrated that cysteamine-HCl caused immediate disruptions to normal gastroduodenal motor activity. Thus, there would appear to be a potential, therapeutic role for drugs which can correct these apparent motor disturbances, and promote normal, coordinated gastroduodenal motility.

The present results provide evidence that a correlation may exist between the effectiveness of a drug in treating duodenal ulcer disease and the number of ulcerogenic factors that it affects. In addition to their profound effects on gastrointestinal motor activity, baclofen, and the GABA antagonists bicuculline and picrotoxinin have the potential to affect acid secretion. The anti-muscarinic actions of U74500A may also affect acid secretion. It would appear that these compounds are as effective or better than cimetidine as anti-duodenal ulcer agents. Cimetidine exerts its effects via inhibition of acid secretion. This suggests that the actions of

cimetidine and baclofen/bicuculline/picrotoxinin/U74500A may be additive. Indeed, it is plausible that no single drug is completely effective and successful in treating this disease. Rather, combination therapy with drugs targeted for motility (ie. Lioresal) and acid secretion (ie. cimetidine) may represent the most suitable approach for duodenal ulceration. These studies should now be extended to examine this notion. Moreover, additional studies need to be undertaken to address the potential mechanism(s) involved in the effects of cysteamine-HCl, including examination of the effects of 1) GABA antagonists (bicuculline and picrotoxinin), 2) baclofen and 3) U74500A on cysteamine-HCl induced disruptions of motility. The effects of cysteamine-HCl on gastroduodenal motility should also be examined to determine if the ulcerogen-induced enhancement of contractile activity is mediated by direct actions on the smooth muscle.

The current data acquisition and data analysis software requires important modifications for future studies investigating gastrointestinal motility. The data acquisition program needs to be converted for direct on-line acquisition and data analysis. This added level of sophistication will remove some of the inefficiency in the present set-up.

The data analysis software should also be modified to allow a more indepth analysis of both individual and propagating motor activity of the gastroduodenum. More specifically, both temporal and spatial relationships of motility, such as the length of spread and velocity of responses needs to be investigated for differentiation between stationary and propagating motor activity. This kind of

analysis will result in further understanding of the role of motility in the control of gastrointestinal function. In addition, pattern-recognition of specific types of motor events, such as 'giant contractions', will allow a better understanding of gastrointestinal motility. Normal motility has to be fully defined before a complete understanding of motility disruptions in the pathogenesis of gastrointestinal diseases can be obtained.

In conclusion, the peripheral GABAergic system appears to function in the modulation of experimental duodenal ulcer, with the neuroendocrine actions of GABA being potentially important to the development of this disease. Thus, peripheral GABAergic receptor sites may represent important new target sites for treatment of duodenal ulcers. Future studies, investigating in greater depth, the extent of involvement of the GABAergic system to duodenal ulceration, will allow a better understanding of this disease. In addition, this work has also demonstrated that the 'lazaroid', U74500A, like baclofen, possesses powerful, anti-duodenal ulcer actions, which may represent another useful treatment for duodenal ulceration. These kinds of studies will eventually allow more appropriate therapies to be initiated for the treatment of duodenal ulcers.

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APPENDIX 1

```

\ Version 06/09/91
\ ACQ005 - Allison E. McKay.
\ -----
\ DATA ACQUISITION PROGRAM FOR GASTROINTESTINAL MOTILITY.
\ Maximum acquisition from 4 channels.
\
\ The status array will store the following information:
\ status [ 1 ] .... #.points
\ status [ 2 ] .... #.channels
\
cr ."Version 6..."
cr
DT2800
token billybob
exp.mem> billybob
integer dim[ 5000 , 4 ] unnamed.array
becomes> billybob
integer
dim[ 50 , 4 ] array buff1
dim[ 50 , 4 ] array buff2
real
scalar conv.del
0.5 conv.del :=
0 3 a/d.template temp
buff1 buff2 cyclic double.template.buffers
vupart vupart1
vupart vupart2
vupart vupart3
vupart vupart4
integer
scalar 1/2
scalar billy.points
scalar #.channels
scalar count
scalar sc#
scalar input.choice \ For the file setup word.
64 string temp.file \ For the temporary data file.
dp.integer
scalar #.points
scalar last.stored \ The last stored point, used when ending.
dim[ 5 ] array status

```

```

1 string del

real
  scalar index.a
  scalar index.b
  scalar index.c .

all.file.clear

\ Close any open files.

?file.open if file.close then
?random.file.open if random.close then

does.file.exist? \ (filename - T/F)
defer> file.sizes \ Take string filename off symbol stack, ask DOS
0 = \ to report total size and number of matches.
0 = \ If any thing is found, file does exist, leave
or not \ true/false on symbol stack for return.
;

: overwrite \ (filename -)
\ Completely overwrite any existing file with same name.
all.file.clear \ Close any open files
"dup does.file.exist? \ Check if exists
if \ If so, DOS will delete it.
  defer> delete
else
  "drop \ Else, drop filename off stack
then
;

: init.tokens
  0 billybob :=
  1 last.stored :=
  1 #.points :=
  1 billy.points :=
  0 status :=
;

: #>"
  32 "compress
;

: yes.or.no
  "input del " :=
  del "N" " = del "Y" " = or
  if
  else
  bell bell
  cr
  "Try again. Y or N?"
  "input del " :=

```

```

then
;

: press.it
" Press <enter> to continue."
key drop
;

: ask.them
begin
cr ." How many data channels do you wish to acquire data from (max. 4)?"
#input not #.channels := #.channels 5 < not or
while
cr cr ." Invalid number. Please enter the # of data channels (max. 4)."
repeat
;

: vuport.1          \ vuport 1-4 set-up
graphics.display
vuport1
9 vuport.color
0.28 vuport.orig
1.72 vuport.size
15 axis.color 14 cursor.color 0 color
.05 .05 axis.orig
.9 .9 axis.size vuport.clear

horizontal grid.off
vertical grid.off
horizontal no.labels
vertical no.labels
.0125 .0125 tick.size
.5 .5 tick.just
normal.coords .5 .5 axis.point
world.coords
vertical 0.5 world.set
20 10 axis.divisions

outline xy.axis.plot
;

: vuport.2
graphics.display
vuport2
4 vuport.color
0.28 vuport.orig
1.36 vuport.size
15 axis.color 0 cursor.color 0 color
.05 .05 axis.orig
.9 .9 axis.size vuport.clear

vuport1
9 vuport.clear
0.64 vuport.orig

```

```

1. .36 vuport.size
15 axis.color 14 cursor.color 0 color
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
horizontal grid.off
vertical grid.off

.0125 .0125 tick.size
.5 .5 tick.just
horizontal no.labels
vertical no.labels
normal.coords .5 .5 axis.point
world.coords
vertical 0. 5. world.set
20 10 axis.divisions

vuport1 outline xy.axis.plot
vuport2 outline xy.axis.plot
;
: vuport.3
graphics.display
vuport3
7 vuport.color
0. .28 vuport.orig
1. .24 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 5 color
.0125 .0125 tick.size
.5 .5 tick.just

vuport2
4 vuport.color
0. .52 vuport.orig
1. .24 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 0 color
.0125 .0125 tick.size
.5 .5 tick.just

vuport1
9 vuport.color
0. .76 vuport.orig
1. .24 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 0 color
.0125 .0125 tick.size
.5 .5 tick.just

horizontal grid.off
vertical grid.off
horizontal no.labels
vertical no.labels
normal.coords .5 .5 axis.point
vertical 0. 5. world.set

```

```

20 10 axis.divisions

vuport1 outline xy.axis.plot
vuport2 outline xy.axis.plot
vuport3 outline xy.axis.plot
;

: vuport.4
graphics.display

vuport4
9 vuport.color
0.82 vuport.orig
1.18 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 0 color

vuport3
4 vuport.color
0.64 vuport.orig
1.18 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 0 color

vuport2
7 vuport.color
0.46 vuport.orig
1.18 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 5 color

vuport1
8 vuport.color
0.28 vuport.orig
1.18 vuport.size
.05 .05 axis.orig
.9 .9 axis.size vuport.clear
15 axis.color 0 cursor.color 14 color

horizontal grid.off
vertical grid.off
horizontal no.labels
vertical no.labels
.0125 .0125 tick.size
.5 .5 tick.just
normal.coords .5 .5 axis.point
world.coords
vertical 0.5 world.set
20 10 axis.divisions

vuport1 outline xy.axis.plot
vuport2 outline xy.axis.plot
vuport3 outline xy.axis.plot
vuport4 outline xy.axis.plot
;

```

```
22 0 25 80 window {prompt}
```

```
: prompt.them
  {prompt}
  screen.clear
  ." Press <A> or <a> to abort from data acquisition."
;
```

```
: set.up.vuports
#channels 1 =
  if
    vuport.1
  then
#channels 2 =
  if
    vuport.2
  then
#channels 3 =
  if
    vuport.3
  then
#channels 4 =
  if
    vuport.4
  then
;
```

```
: set.up.temp.file
```

```
\ Set up and initialize a temporary storage file for the data.
\ Note that if you simply enter a filename.ext, it will go on
\ the default subdirectory and drive.
```

```
begin
```

```
cr ." Please enter the temp. data storage filename. Up to 64 characters"
cr ." can be entered, in the form of drive:\path\filename.ext. Default"
cr ." path is : ".dir
cr ." Your choice: "
"input temp.file " :=
temp.file
does.file.exist?
if
```

```
cr ." A file by that name already exists. You can choose to:"
cr ." 1. Select another name"
cr ." 2. Overwrite it and continue"
cr ." 3. Exit to the OK prompt"
cr ." Decisions, decisions.... (1,2,3) : "
```

```
0 input.choice := \ Reset
#input \ Get input
if \ If valid number,
  input.choice := \ set here,
  input.choice 2 =
  if
    temp.file \ Erase filename from disk.
    overwrite
  else
    input.choice 3 = \
```

```

        if quit \ Exit to OK prompt
        then
        then
        then
input.choice 2 = \ The only valid way out.
else
true \ File did not exist, so continue
then

until \ Only ends if:
      \ file doesn't exist, or
      \ file did exist, but was over-
      \ written in choice #2.

file.template
5 comments
dp.integer dim[ 5 ] subfile
end
temp.file "dup
defer> file.create \ Create and open file
defer> file.open

cr ." Experiment #: " \ Important questions.
"input
1 >comment
cr ." Rat #: "
"input
2 >comment
cr ." VHS Tape #: "
"input
3 >comment
cr ." Treatment: "
"input
4 >comment

\ Leave file open...
;

: close.temp.file

#.points
status [ 1 ] :=
#.channels
status [ 2 ] :=
1 subfile
status array > file

file.close

;

: set.up.tasks

init.tokens \ Initalize variables.

```

```

das.init
clear.tasks
50 task.period

temp 1 task a/d.in > array
4 1 task.modulo

conv.del conversion.delay
a/d.init
prime.tasks
;

: store.some.data

\ This word gets called once every 5000 points, and is the data storage
\ word. It also gets called at the end of the sampling, only if the
\ number of data points in #.POINTS is greater than LAST.STORED.

billybob
sub[ 1 5000 ; 1 #.channels ]
append.array > file
last.stored 5000 +
last.stored :=
;

: plot.this.instead

#.channels 1 + 1 do

  " vuport"
  i ." 32 "compress
  "cat
  "exec

  real 50 ramp #.points +

  1/2 0 =
  if
    buff1
  else
    buff2
  then
    xsect[!,i]
    5. * 1024. / neg 5. +

  #.points 500 modulo
  1 <>
  if
    world.coords
    dup [ 1 ]
    #.points
    swap draw.to
  then
    xy.data.plot

loop
;

```

more.data.now

```
#.points 500 modulo
1 =
if
#.channels 1 + 1 do
"vuport"
i " " 32 "compress
"cat
"exec
vuport.clear
horizontal
world.coords
axis.fit.off
#.points 1 -
dup 500 +
world.set
xy.axis.plot loop
then
```

```
1/2 0 =
if
buff1
else
buff2
then
```

```
billybob sub[ billy.points , 50 ; 1 , 4 ] :=
plot.this.instead
```

```
#.points 50 + \ Adjust offset (ie. counter) for indicating 50 more points
#.points := \ for each data channel.
```

```
billy.points 50 +
billy.points :=
billy.points 5000 >
if
store.some.data
1 billy.points :=
then
;
```

```
: test.it
```

```
1 #.points :=
0 1/2 :=
trigger.tasks
```

```
begin
```

```
1/2
```

```
?buffer.a/b
```

```
if
0 =
if
```

```

        more.data.now
        1 1/2 :=
    then
else
    1 =
    if
        more.data.now
        0 1/2 :=
    then
    then

false
?key
if
    pkey
    not if
        dup 65 =
        97 = or
        if
            ?drop
            true
        then
    then
    then

#.points 50000 >
or
until
;

: stop.acq
    stop.tasks
    clear.tasks

    \ Check to determine if there is any data remaining.

#.points last.stored >
if
    store.some.data
then

close.temp.file
;

: first.things.first
0 count =
if
    set.up.vuports
    prompt.them
then
;

: Try.it
ask.them
first.things.first
set.up.temp.file
set.up.tasks
test.it

```

stop.acq

252

```
cr ." To run the program, type 'try.it'... "  
cr
```

APPENDIX 2

```
/* gi2.c
```

```
Frank Johnson
```

```
30 November 1991
```

```
Program to import gi data and perform contraction/relaxation analysis
```

```
*/
```

```
#include <dos.h>
#include <stdio.h>
#include <conio.h>
#include <graphics.h>
#include <math.h>
#include <ctype.h>
#include <mem.h>
#include <process.h>
#include <stdlib.h>
```

```
#include "gi2.h"
```

```
char Inname[32], Outname[32];
char dump[8192];
char outstr[90];
char instr[42];
```

```
#define maxchan 8
```

```
char coltbl[maxchan] = {2,3,4,5,6,7,8,1};
```

```
typedef struct
```

```
{
float a0;
float a1;
char calunits[16];
char frmt[16];
int dsp;
int dsph;
} calstruct;
```

```
calstruct calib [maxchan];
```

```
avdata [500][maxchan];
```

```
FILE *InFile, *CalibFile, *ParamFile, *OutFile;
int outfileflag=0;
int i,j,z;
```

```
long int nfileread;
```

```
int chan,nhead=1536,nperiod=300,nsec=60;
```

```
int gd,gm;
float rate1=20.0,rate2=300.0; /* rates for l.p. filters */
```

```

int p1,p2,p3,p4,p5,p6,pf1,pf2,tp1,tp2;
int n1,n2,n3,n4,n5,n6,nf1,nf2,tn1,tn2;

/***** declarations for data buffer *****/
typedef struct
{
  int *head;
  int *tail;
  int *bbuf;
  int *ebuf;
} QUEUE;

QUEUE dq;

#define RQS 600 /* 600 = two minutes at 200 msec samples */

int rd[RQS]; /* queue buffer */

/* variables for positive peak search */

int Aflag,Bflag,tp,Alev,parea,pkfound;
int maxlev=-2048,maxflg;

int Dflag,Eflag,tn,Dlev,narea;
int minlev=2048,minflg;

#define flushstdin while(getchar()!='\n'); /* to remove \n after scanf */

void main()
/*****/
{
  char c,quit=0;

  detectgraph(&gd,&gm);
  initgraph(&gd,&gm,"");

  restorecrtmode();

  read_calib();

  clrscr();

  show_menu();

  do
  {
    c = toupper(getch());

    switch(c)
    {
      case 'A' : analyse();break;
      case 'C' : channel_select();break;
      case 'D' : break;
      case 'F' : file_select();break;
      case 'H' : enter_header();break;
      case 'L' : low_freq_analyse();break;
      case 'O' : output_file();break;
    }
  }
}

```

```

    case 'P' : enter_period();break;
    case 'Q' : quit=1;break;
    case 'S' : segment_display();break;
    case 'R' : relax_con();break;
    case 'U' : param_enter();break;
    default : show_menu();
}
}while(quit==0);

fclose(InFile);
closegraph();
}

void dumscanf()
/*****/
{float r;
scanf("%f",&r);}

void read_calib()
/*****/
{
int ichan;

if((CalibFile=fopen("gi2.cal","r"))==NULL)
{
printf("Unable to open calibration file - using default values:\n");
delay(500);
}
else
{
for (ichan=0;ichan<maxchan;ichan++)
{
fscanf(CalibFile,"%f %f %s %s %d %d",
&calib[ichan].a0,&calib[ichan].a1,calib[ichan].calunits,calib[ichan].frmt,
&calib[ichan].dspl,&calib[ichan].dsph);
}
}

if((ParamFile=fopen("gi2.prm","r"))==0)
{
printf("Unable to open parameter file - enter new values: \n");
param_enter();
}
else
{
fgets(instr,81,ParamFile); /* ignore first line */
fscanf(ParamFile,"%d %d %d %d %d %d %d %d %d %d %d %d",
&p1,&n1,&p2,&n2,&p3,&n3,&p4,&n4,&p5,&n5,&p6,&n6);
}
}

}

void show_menu()
/*****/
{
restorecrtmode();
clrscr();
}

```

```

gotoxy(30,1);printf("  G-I Data Analysis");
gotoxy(30,2);printf("Ottawa Instrumentation Ltd.");

gotoxy(1,4);printf("Menu options: ");

gotoxy(10,wherey()+1); printf("A analyse");
gotoxy(10,wherey()+1); printf("C channel select      %d",chan+1);
gotoxy(10,wherey()+1); printf("F file select      %s",Inname);
gotoxy(10,wherey()+1); printf("H header specify  %d",nhead);
gotoxy(10,wherey()+1); printf("L low frequency analysis");
gotoxy(10,wherey()+1); printf("O Output file:    %s",Outname);
gotoxy(10,wherey()+1); printf("P averaging period entry %d",nsec);
gotoxy(10,wherey()+1); printf("Q quit");
gotoxy(10,wherey()+1); printf("S segment display");
gotoxy(10,wherey()+1); printf("R analyse relaxation/contractions");
gotoxy(10,wherey()+1); printf("U Relaxation/contraction setup ");
}

```

```

void file_select()
/*****/
{
fclose(InFile);
restorecrtmode();
gotoxy(1,20);
printf("\n\nEnter file name : ");
scanf("%s",Inname);

if((InFile=fopen(Inname,"rb")) == NULL)
{
printf("\n\n -- no file %s found ",Inname);
}
else
{
printf("\n input file %s found ",Inname);
}
}

```

```

void output_file()
/*****/
{
fclose(OutFile);
outfileflag=0;
restorecrtmode();
gotoxy(1,20);
printf("\n\nEnter output file name : ");
scanf("%s",Outname);

if((OutFile=fopen(Outname,"r")) == NULL)
{
OutFile=fopen(Outname,"w");
outfileflag=1;
}
else
{
printf("\n\n -- file already exists !!");
}
}

```

```

void channel_select()
/*****/
{
    restorecrtmode();
    gotoxy(1,20);
    printf(" Enter channel [1..3] ");
    scanf("%d",&chan);
    if ((chan < 1) || (chan > 3)) chan = 0; else chan--;
}

void enter_header()
/*****/
{
    restorecrtmode();
    gotoxy(1,20);
    printf(" Enter number of bytes of header [%d] ",nhead);
    scanf("%d",&nhead);
}

void enter_period()
/*****/
{
    restorecrtmode();
    gotoxy(1,20);
    printf(" Enter averaging period [%d seconds] ",nsec);
    scanf("%d",&nsec);
    nperiod = nsec*5;          /* expect five samples /second */
}

void analyse()
/*****/
{
    int navg;

    navg = 0;

    /* fread(dump,nhead,1,InFile); */
    /* dump header information */
    fseek(InFile,nhead,SEEK_SET);

    write_axes();

    sprintf(outstr,"%4.0f min",nsec*250./60.);
    outtextxy(505,460,outstr);

    draw_legend();

    nfileread = 0;

    if(outfileflag)fprintf(OutFile,"Analysis output for %s, period %d\n\n",Inname,nsec);

    do
    {
        compute_averages(navg);
        plot_averages(navg);
    }
}

```

```

    navg+ +;
} while ((!feof(lnFile)) && (navg < 500));
rewind(lnFile);
}

void write_axes()
/******/
{
int i;

setgraphmode(gm);
setcolor(7); /* select colour of axes */

line(0,120,639,120); /* horizontal axes */
line(0,360,639,360);

setcolor(15);

for (i=0;i<10;i+ +) /* vertical axis */
{
line(0,i*48,10,i*48);
line(0,i*48,0,i*48+48);
}
for (i=0;i<10;i+ +) /* horizontal axis */
{
line(i*64,479,i*64,470);
line(i*64,479,i*64+64,479);
}
}

void draw_legend()
/******/
{
int xpos,ypos;

ypos=10; xpos=500;
setcolor(15);
setusercharsize(1,1,1,1);
settextstyle(0,0,0);

setcolor(coltbl[0]); sprintf(outstr,"%s",calib[0].calunits);
outtextxy(18+xpos,10+ypos,outstr);

setcolor(coltbl[1]); sprintf(outstr,"%s",calib[1].calunits);
outtextxy(18+xpos,20+ypos,outstr);

setcolor(coltbl[2]); sprintf(outstr,"%s",calib[2].calunits);
outtextxy(18+xpos,30+ypos,outstr);

setcolor(coltbl[3]); sprintf(outstr,"%s",calib[3].calunits);
outtextxy(18+xpos,40+ypos,outstr);

setcolor(coltbl[4]); sprintf(outstr,"%s",calib[4].calunits);
outtextxy(18+xpos,50+ypos,outstr);

setcolor(coltbl[5]); sprintf(outstr,"%s",calib[5].calunits);
outtextxy(18+xpos,60+ypos,outstr);
}

```

```

setcolor(coltbl[6]); sprintf(outstr,"%s",calib[6].calunits);
outtextxy(18+xpos,70+ypos,outstr);

setcolor(coltbl[7]); sprintf(outstr,"%s",calib[7].calunits);
outtextxy(18+xpos,80+ypos,outstr);
}

void compute_averages(int na)
/*****
{
int iavg,min,max,s_min,s_max,slope;
int indata[3];
float realavg,slopeavg,tot_period;

min = s_min = +8192;
max = s_max = -8192;

realavg = slopeavg = iavg = tot_period = 0;

do
{
fread(indata,3,2,InFile);

if((iavg==0)&&(nfileread==0)) indata[0] = 512; /* drop 1st value */

nfileread++;
if((nfileread%5000)==0)
{
fseek(InFile,80,SEEK_CUR); /* skip next 80 bytes */
nfileread=0;
}

realavg += indata[chan]; /* mean */
min = (min < indata[chan]) ? min : indata[chan];
max = (max > indata[chan]) ? max : indata[chan];
tot_period += compute_period(indata[chan]);

slope = diff(indata[chan]);
slopeavg += abs(slope);

s_min = (s_min < slope) ? s_min : slope;
s_max = (s_max > slope) ? s_max : slope;

iavg++;
} while(iavg < nperiod);

avdata[na][0] = realavg/iavg-512;
avdata[na][1] = min - avdata[na][0]-512;
avdata[na][2] = max - avdata[na][0]-512;
avdata[na][3] = slopeavg/iavg;
avdata[na][4] = s_min;
avdata[na][5] = s_max;
/*
avdata[na][6] = (tot_period!=0) ? 300/(tot_period/iavg) : 480; /* cycles/min */
*/
avdata[na][6] = tot_period/iavg;
}

```

```

void plot_averages(int na)
/******/
{
int yoff,ys,xs,j;

xs = 2;

if(na < 1) return;

for(j=0;j < maxchan;j++)
{
switch(j)
{
case 0 : yoff=240; ys=5; break;
case 1 :
case 2 : yoff=120; ys=5; break;

case 3 : yoff=360; ys=5; break;
case 4 :
case 5 : yoff=360; ys=20; break;

case 6 : yoff=480; ys=5; break;

default : yoff=240; ys = 1; break;
}

setcolor(coltbl[j]);
line((na-1)*xs,yoff-avdata[na-1][j]/ys,na*xs,yoff-avdata[na][j]/ys);
if(outfileflag) fprintf(OutFile, "%6d", avdata[na][j]);
}
if(outfileflag) fprintf(OutFile, "\n");
}

int diff(int nval)
/******/
{
static int n0,n1,n2,n3,n4,n5,n6,n7,n8,n9;

n9=n8;n8=n7;n7=n6;n6=n5;n5=n4;n4=n3;n3=n2;n2=n1;n1=n0;n0=nval;

return(n0+2*n1+4*n2+2*n3+n4-n5-2*n6-4*n7-2*n8-n9);
}

void segment_display()
/******/
{
int offset,ldata[3],ldata[3],yoff,ngap;
long int nx;
float seglen;

gotoxy(1,20);
printf("Enter offset into file (minutes) and segment length: ");

scanf("%d %f",&offset,&seglen);

if(seglen == 0) seglen = 6.4;

```

```

ngap = (int) offset*60l*5/5000;    /* number of 5000 sample blocks */
if(fseek(InFile,offset*6l*60*5+nhead+ngap*80,SEEK_SET) == 0)
{
    nx = 0;

    write_axes();
    sprintf(outstr,"%4.1f min from %d min",seglen,offset);
    outtextxy(450,460,outstr);

    nfileread = (int) offset*60l*5 % 5000;    /* samples read into block */
    do
    {
        fread(indata,3,2,InFile);
        nfileread++;
        if((nfileread%5000) == 0)
        {
            fseek(InFile,80,SEEK_CUR);    /* skip next 80 bytes */
            nfileread=0;
        }

        for(i=0;i<3;i++)
        {
            yoff = i*120+240;

            setcolor(coltbl[i]);
            if(nx>0)line(nx/(3*seglen/6.4),yoff-ldata[i]/5,nx/(3*seglen/6.4),yoff-indata[i]/5);
            ldata[i] = indata[i];
        }
        nx++;
    }while(nx<(seglen*300));
}
rewind(InFile);
}

void low_freq_analyse()
/*****/
{
    int offset,yoff,ngap,indata[3];
    long int nx;
    float seglen;
    int inchan,ifil1,ifil2,ldata,lfil1,lfil2,iperiod,lperiod;
    float fil1,fil2;

    gotoxy(1,20);
    printf("For low frequency analysis: offset & seglen (min) ");

    scanf("%d %f",&offset,&seglen);
    printf("enter filter rates 1 [%5.0f] and 2 [%5.0f] : ",rate1,rate2);
    scanf("%f %f",&rate1,&rate2);

    if(seglen==0)seglen = 6.4;

    ngap = (int) offset*60l*5/5000;    /* number of 5000 sample blocks */
    if(fseek(InFile,offset*6l*60*5+nhead+ngap*80,SEEK_SET) == 0)
    {

```

```

nx = 0; lperiod = 0;

write axes();
sprintf(outstr,"chan %d & periods: %4.1f min from %d min",chan+1,seglen,offset);
outtextxy(350,460,outstr);

nfileread = (int) offset*60l*5 % 5000; /* samples read into block */
do
{
fread(indata,3,2,InFile);
inchan = indata[chan]-512; /* pick up current channel */
if(nx==0)
{
fil1 = fil2 = inchan;
}

nfileread++;
if((nfileread%5000) == 0)
{
fseek(InFile,80,SEEK_CUR); /* skip next 80 bytes */
nfileread=0;
}

fil1 = (fil1*(rate1-1) + inchan)/rate1; ifil1 = fil1;
fil2 = (fil2*(rate2-1) + inchan)/rate2; ifil2 = fil2;

if((ifil1 > ifil2)&&(lfil1 <= lfil2))
{
lperiod = nx-lperiod;
lperiod = nx;
}
yoff = 240;

if(nx > 0)
{
setcolor(coltbl[chan]);
line(nx/(3*seglen/6.4),yoff-ldata/2,nx/(3*seglen/6.4),yoff-inchan/2);
setcolor(coltbl[chan+1]);
line(nx/(3*seglen/6.4),yoff-lfil1/2,nx/(3*seglen/6.4),yoff-ifil1/2);
setcolor(coltbl[chan+2]);
line(nx/(3*seglen/6.4),yoff-lfil2/2,nx/(3*seglen/6.4),yoff-ifil2/2);
setcolor(coltbl[chan+3]);
line(nx/(3*seglen/6.4),480-lperiod,nx/(3*seglen/6.4),480-lperiod);

ldata = inchan; lfil1 = ifil1; lfil2 = ifil2;
}
nx++;
}while(nx < (seglen*300));
}

rewind(InFile);
}

int compute_period(int data_val)
/*****/
{
int ifil1,ifil2;

```

```

static int lperiod,period,lfil1,lfil2;
static float fil1,fil2;

fil1 = (fil1*(rate1-1)+data_val)/rate1; ifil1 = fil1;
fil2 = (fil2*(rate2-1)+data_val)/rate2; ifil2 = fil2;

if((ifil1 > ifil2)&&(lfil1 <= lfil2))
{
    period=lperiod;
    period=0;
}
period++;
lfil1 = ifil1; lfil2 = ifil2;
return(period);
}

void relax_con()
/*****/

/* will analyse the current channel for contractions/relaxations,
and output a stream of data giving the mean contraction and mean relaxation
for each epoch
*/

/* any event in progress will be rolled into the next epoch */
{
int np,pp;
int offset,ngap,nx,indata[3],inchan,yoff,ldata,iavg;
float seglen,totparea,totnarea;

gotoxy(1,20);
printf("For relaxation/contraction analysis: offset & seglen (min) ");
scanf("%d %f",&offset,&seglen);
if(seglen==0) return; /* offer a way out */
totparea = totnarea = 0;   iavg = 0;
initq(rd,RQS); /* initialize ring buffer for detector */
inita(); /* and analysis variables */
ngap = (int) offset*60*5/5000; /* number of 5000 sample blocks */
yoff = 240;
if(fseek(InFile,offset*60*5+nhead+ngap*80,SEEK_SET) == 0)
{
    nx = 0;

    write_axes();
    sprintf(outstr,"chan %d & r/c: %4.1f min from %d min",chan+1,seglen,offset);
    outtexby(350,460,outstr);

    if(outfileflag)
    {
        fprintf(OutFile,"Relax/con analysis for file %s. Period: %d sec\n",Inname,nsec);
        fprintf(OutFile,"channel %d & r/c: %4.1f min from %d min\n\n",chan+1,seglen,offset)
    }
}
}

```

```

}
nfileread = (int) offset*60l*5 % 5000; /* samples read into block */
do
{
fread(indata,3,2,InFile);
iavg++;
inchan = indata[chan]-512; /* counter for output */
/* pick up current channel */

to_filobuf(inchan);

nfileread++;
if((nfileread%5000) == 0)
{
fseek(InFile,80,SEEK_CUR); /* skip next 80 bytes */
nfileread=0;
}

if(nx>0)
{
setcolor(coltbl[chan]);
line(nx/(3*seglen/6.4),yoff-ldata/2,nx/(3*seglen/6.4),yoff-inchan/2);

pp=ppeak(inchan);
setcolor(coltbl[chan+1]);
if(Aflag)putpixel(nx/(3*seglen/6.4),yoff-Alev/2+20,coltbl[chan+1]);
if (pp>0)
{
totparea += pp;
line(nx/(3*seglen/6.4),yoff-inchan/2+20,nx/(3*seglen/6.4),yoff-inchan/2+40);
}

np=npeak(inchan);
setcolor(coltbl[chan+2]);
if(Dflag)putpixel(nx/(3*seglen/6.4),yoff-Dlev/2+60,coltbl[chan+2]);
if (np<0)
{
totnarea -= np;
line(nx/(3*seglen/6.4),yoff-inchan/2+60,nx/(3*seglen/6.4),yoff-inchan/2+80);
}
}
data = inchan;
}
nx++;
if(iavg == nperiod)
{
settextstyle(0,1,1);
sprintf(outstr,"Tot p %6.0f n %6.0f",totparea,totnarea);
outtextxy(nx/(3*seglen/6.4),0,outstr);
if(outfileflag)fprintf(OutFile,"p %6.0f n %6.0f\n",totparea,totnarea);
iavg=0;
settextstyle(0,0,1);
totparea=totnarea=0;
}
}while((nx < (seglen*300))&&(!feof(InFile)));
rewind(InFile);
}

```

```
int ppeak(int inval)
```

```

/*****/
{
int ais;          /* positive slope */
    tp ++;
    ais = inval-filobuf(p3);
    if((ais>p1)&&(Aflag==0)&&(Dflag==0)) /* looking for start */
    {
        Aflag = 1;
        tp = 0;
        Alev = inval;
        parea = 0;
    }

    if(Aflag) parea += inval - Alev;      /* increment area count */
    if (Aflag&&(Bflag==0)&&(tp>p4)&&(tp<p5)) /* looking for peak */
    {
        Bflag=find_max(inval);
    }

    if((Aflag)&&(tp>p5)) /* gone too far */
    {
        Aflag = 0; /* stop looking */
        parea = 0; /* clear area */
    }

    if(Aflag&&Bflag
        &&((inval-Alev)<p6)) /* back to baseline ? */
    {
        /* found a contraction */
        Aflag = 0;
        Bflag = 0;
        return(parea);
    }
    return(0);
}

int find_max(int val)
/*****/
{
    if(val>maxlev)
    {
        maxlev = val;
        maxflg = 1;
    }
    if ((maxflg==1)&&((maxlev-val)>p2))
    {
        maxlev = -2048;
        return(1);
    }
    return(0);
}

int npeak(int inval)

```

```

/*****/
{
int aes;          /* negative slope */
    tn++;
    aes = filobuf(n3)-inval;
    if((aes>n1)&&(Dflag==0)&&(Aflag==0)) /* looking for start */
    {
        Dflag = 1;
        tn = 0;
        Dlev = inval;
        narea = 0;
    }
    if(Dflag) narea += inval - Dlev;    /* increment area count */
    if (Dflag&&(Eflag==0)&&(tn>n4)&&(tn<n5)) /* looking for trough */
    {
        Eflag=find_min(inval);
    }
    if((Dflag)&&(tn>n5)) /* gone too far */
    {
        Dflag = 0;    /* stop looking */
        narea = 0;    /* clear area */
    }
    if(Dflag&&Eflag
        &&((Dlev-inval)<n6)) /* back to baseline ? */
    {
        /* found a relaxation */
        Dflag = 0;
        Eflag = 0;
        return(narea);
    }
    return(0);
}

int find_min(int val)
/*****/
{
    if(val<minlev)
    {
        minlev = val;
        minflg = 1;
    }
    if ((minflg==1)&&((val-minlev)>n2))
    {
        minlev = 2048;
        return(1);
    }
    return(0);
}

int filobuf(int index)
/*****/
{

```

```

int *p,i;

    p = dq.tail;
    p = p-index;
    if(p < dq.bbuf)
    {
        i = dq.ebuf-dq.bbuf;
        p=p+i;
    }
    return(*p);
}

void to_filobuf(int num)
/******/
{
int *p;

    p = dq.tail;
    *p = num;
    p++;
    if(p == dq.ebuf) p = dq.bbuf;
    dq.tail = p;
}

void initq(int *buf,int nbuf)
/******/
{
    dq.head = buf;
    dq.tail = buf;
    dq.bbuf = buf;
    dq.ebuf = buf+nbuf;
}

void inita()
/******/
{
    Aflag = 0;
    Bflag = 0;
    tp = 0;
    parea = 0;
}

void param_enter()
/******/

/* parameters following Schemann, with extra flags and times: */

/*
int p1,p2,p3,p4,p5,p6;          Defined as globals
int n1,n2,n3,n4,n5,n6;
*/

{

char c,qflag;
int  xdlog,ydlog;

    xdlog = 1;
    ydlog = 20;
}

```

```

clrscr();

ShowParmMenu();
while(kbhit())getch();
qflag = 1;

while(qflag)
{
gotoxy(xdlog,ydlog);clreol();
c = toupper(getch());
switch(c)
{

case '1' : gotoxy(xdlog,ydlog);
printf("p1,n1 rise [%d %d a/d units] ",p1,n1);
scanf("%d %d",&p1,&n1);flushstdin
ShowParmMenu();
break;

case '2' : gotoxy(xdlog,ydlog);
printf("p2,n2 fall [%d %d a/d units] ",p2,n2);
scanf("%d %d",&p2,&n2);flushstdin
ShowParmMenu();
break;

case '3' : gotoxy(xdlog,ydlog);
printf("p3,n3 period [%d %d samples] ",p3,n3);
scanf("%d %d",&p3,&n3);flushstdin
ShowParmMenu();
break;

case '4' : gotoxy(xdlog,ydlog);
printf("p4,n4 period [%d %d samples] ",p4,n4);
scanf("%d %d",&p4,&n4);flushstdin
ShowParmMenu();
break;

case '5' : gotoxy(xdlog,ydlog);
printf("p5,n5 timeout [%d %d samples] ",p5,n5);
scanf("%d %d",&p5,&n5);flushstdin
ShowParmMenu();
break;

case '6' : gotoxy(xdlog,ydlog);
printf("p6,n6 fall [%d %d a/d units] ",p6,n6);
scanf("%d %d",&p6,&n6);flushstdin
ShowParmMenu();
break;

case 'Q' : gotoxy(xdlog,ydlog);
printf("Write to file ? ");
c = 'q';
while(!((is_in(c,"YN")))) c = toupper(getche());
if(c == 'Y')
{
ParamFile = fopen("G12.Prm","w");
fprintf(ParamFile,"New Parameters for gi2 \n");
}
}
}

```

```

        fprintf(ParamFile,"%d %d %d %d %d %d %d %d %d %d %d %d",
        p1,n1,p2,n2,p3,n3,p4,n4,p5,n5,p6,n6);
    }
    qflag = 0;
    break;
}
}
}

void ShowParmMenu()
/*****/
{
    gotoxy(10,2); printf("Parameters for analysis: \n\n");
    gotoxy(10,wherey()+1);printf("1 baseline rise %5d %5d",p1,n1);
    gotoxy(10,wherey()+1);printf("2 peak fall %5d %5d",p2,n2);
    gotoxy(10,wherey()+1);printf("3 baseline time %5d %5d",p3,n3);
    gotoxy(10,wherey()+1);printf("4 min peak time %5d %5d",p4,n4);
    gotoxy(10,wherey()+1);printf("5 max peak time %5d %5d",p5,n5);
    gotoxy(10,wherey()+1);printf("6 return level %5d %5d",p6,n6);
    gotoxy(10,wherey()+2);printf("Q to save and quit");
}

int is_in(char c, char *s)
/*****/
{
    while(*s) if(*s++ == c) return 1;
    return 0;
}

```