IMIDAZOLINE RECEPTOR MODULATION OF GASTRIC ACID SECRETION AND EXPERIMENTAL GASTRIC MUCOSAL INJURY IN RATS

BY

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IMIDAZOLINE RECEPTOR MODULATION OF GASTRIC ACID SECRETION AND EXPERIMENTAL GASTRIC MUCOSAL INJURY IN RATS

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

...But on you will go
though the weather be foul.
On you will go
though your enemies prowl.
On you will go
though the Hakken-Kraks howl.
Onward up many
a frightening creek,
though your arms may get sore
and your sneakers may leak...

- Dr. Seuss -

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ABSTRACT

Epidemiological data indicate an association between portal hypertension and peptic ulcer disease in man. Laboratory data have shown certain antihypertensive agents to be antisecretory and gastroprotective in some experimental paradigms. New antihypertensives that are the target of novel imidazoline receptors, have been shown to affect gastrointestinal function (Glavin and Smyth, 1995). We tested moxonidine (an I_1-imidazoline receptor agonist), and efaroxan (an I_1-imidazoline receptor antagonist), in models of gastric acid secretion and experimental gastric mucosal injury in rats, in order to more fully characterize a role for the imidazoline receptor in mediating gastrointestinal physiology. Following drug administration, gastric acid secretions were collected via chronic indwelling gastric cannulae for determination of gastric acid output. Moxonidine (icv) attenuated basal acid output up to 38%. Pretreatment with efaroxan, blocked moxonidine's antisecretory effects. When administered prior to 3 hr of cold restraint stress, moxonidine (ip) significantly increased gastric adherent mucus levels, an effect which was also antagonized by efaroxan pretreatment. When administered as the sole experimental agent (ip and icv), efaroxan had no effect on either gastric acid output or adherent mucus levels. Agmatine (decarboxylated l-arginine), has been recently identified as a clonidine-displacing substance, and a putative endogenous ligand of the imidazoline receptor. Given moxonidine's gastroprotective capability, we tested agmatine in each of our experimental protocols. Agmatine administration (ip or icv) augmented basal gastric acid secretion to a maximum of 40% and 44% respectively. In animals exposed to cold restraint stress, agmatine decreased gastric adherent mucus and

exacerbated stress-induced gastric mucosal injury. Agmatine was also shown to increase pepsin and acid output in anesthetized rats. Although agmatine has been found in high concentrations within stomach tissue, our laboratory observed that administration of the arginine decarboxylase inhibitor difluoromethylarginine (DFMA) (ip and icv), had no effect on acid secretion. These data showing diametrically opposed gastrointestinal effects for moxonidine and agmatine are congruent with previous findings with blood pressure regulation, which showed a pressor effect of agmatine. The nature of endogenous agonist binding at the I_1-imidazoline receptor site therefore, requires further exploration. Our data cannot exclude the possibility that agmatine may be acting as an 'inverse agonist' at I_1-imidazoline receptors. We conclude that activation of peripheral I_1-imidazoline sites by the selective I_1-agonist moxonidine is associated with potent gastric effects, and may provide a novel mechanism of gastroprotection.

ACKNOWLEDGEMENTS

I first met Dr. Gary Glavin in January of 1994 when I offered to volunteer in his lab for some research experience. And what an experience it's been!! I'll always respect him as a scientist and as a teacher, and I'm grateful that he let me generate and test out, my own research ideas. In having to turn this thesis into a document for the ages, I discovered something about myself that may surprise a few people - I enjoy doing research. I think this came about partly because Dr. Glavin allowed me the freedom to explore intellectual avenues at my own pace.

It may have taken me a little longer than most, to choose a career path. However, there were several members of the department who, because of their dedication, intellectual abilities and professionalism, inspired me. Thank you to Dr. Clive Greenway, Dr. Don Smyth, Dr. Deepak Bose and Dr. Fred Aoki.

Before I forget, I don't think I ever once filled-out one of those professor-evaluation forms. I just thought I'd let you all know that the quality of teaching I received from the various members of the department in the past two years, has been excellent. I'd also like to take this opportunity to apologize to all those faculty, staff and students in the department who may have had the misfortune of having to cross paths with me around exam time.

I must make special mention of Ms. Arleen Hall, as my gratitude to her is unmeasurable. I appreciated her patience when I was learning, her understanding when I was stressed-out, and most of all, her friendship when I needed it most.

Most importantly, I'd like to thank my mother. I shared with her my hardship. Now I want to share with her my achievement. Although I'm not very good at expressing it, this is the perfect time to let her know that I really have appreciated everything that she's done for me. I know that I made it part of the way on my brains and good looks...but the rest of the way, I owe to her love and support.

And finally to all the vast multitudes of friends and well-wishers who sustained me throughout this endevour, I leave you with the sage words of Van Morrison:

...Just to be hep...Get wet...With the jet set...

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REVIEW OF THE LITERATURE

I) PEPTIC ULCER DISEASE

Gastric and duodenal peptic ulcers are inflammatory lesions of the gastric and duodenal mucosae respectively, which penetrate the muscularis mucosa. A number of factors have been shown to contribute to the development of peptic ulcer disease. Most commonly, these include Helicobacter pylori (H. pylori) infection, ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), and physical trauma associated with severe burns, head injury, or major surgery. Gastric and duodenal ulcers are therefore, multifactoral, pluricausal diseases. Ultimately however, lesions arise because the balance of aggressive and defensive factors which normally acts to maintain mucosal integrity, is disrupted.

Although both duodenal and gastric ulcer disease can be characterized by symptoms which may be described as 'typical', a patient may present with gastrointestinal complaints that are not necessarily consistent with the classic symptomatology (Table 1). Generally however, the hallmark features of duodenal ulcer are severe epigastric pain occuring 1 to 3 hours after eating, which can be relieved by eating. Often the pain occurs at night, reflecting the supine position of the patient at bedtime as well as the circadian nature of acid secretion. Weight loss, and nausea and vomiting are also common symptoms of duodenal ulcer. In contrast, gastric ulcer patients are more likely to experience pain sooner after eating, and are less likely to find relief by eating. The extent to which the pain is alleviated following ingestion of antacids is highly variable in ulcer patients and is not a good predictor of ulcer type present (duodenal or gastric). In some individuals ulcers may recur without accompanying symptoms (Jorde et al., 1986), with the possibility that lesions may go

Patient Symptom	Gastric Ulcer (%)	Duodenal Ulcer (%)	Nonulcer Dyspepsia (%)
Pain /discomfort	100	100	100
Epigastric pain	67	61-86	52-73
Frequently severe	68	53	37
Pain occurs within 30 min of eating	20	5	32
Pain increased by food	24	10-40	45
Episodic pain (followed by pain free periods)	16	56	35
Relieved by alkali	36-87	39-86	26-75
Food relief	2-48	20-63	4-32
Occurs at night	32-43	50-88	24-32
Not related to food	22-53	21-49	22-65
Anorexia	46-57	25-36	26-36
Weight loss	24-61	19-45	18-32
Vomiting	38-73	25-57	26-34
Heartburn	19	27-59	28

Table 1: Frequency of Symptoms Reported by Patients with Gastric and Duodenal Ulcers and Nonulcer Dyspepsia

(Adapted from Soll, 1993)

unnoticed until the patient presents with hemorrhage or perforation.

Although multiple lesions may be present in 5 to 20% of peptic ulcer patients, chronic ulcers usually occur as single lesions, with successive layers of fibrinoid necrotic and fibrotic tissue underlying the ulcer crater (Soll, 1993). Diffuse antral gastritis accompanies most peptic ulcers, with active inflammation more severe in gastric than duodenal ulcer (Schrager et al., 1967). This inflammation has since been linked to the presence of antral H. pylori infection, as a positive correlation exists between antral gastritis and bacterial colonization. Acute ulcers, associated with NSAID use or physiological stress (such as patients in an intensive care unit), are usually multiple in number and shallow. There is also less inflammation and fibrosis in the surrounding tissue as compared to a chronic lesion.

Within developed countries, data indicate that the lifetime prevalence of peptic ulcer disease is about 10%, and at any given time, approximately 1% of the population is suffering from active ulcer disease (Soll, 1993). The annual incidence of peptic ulcer disease is estimated to be between 15 and 30 new cases per 1000 individuals. Each year in the United States there are approximately 300,000 new cases of duodenal ulcer, 3.2 million recurrences, and 3000 deaths (Kurata and Haile, 1984). Although males once comprised the majority of ulcer patients, sex differences in ulcer prevalence have been largely obviated due primarily to declining rates in younger men and increasing rates in older women (Kurata et al., 1985). The two diseases differ in their age of onset. Duodenal lesions usually occur for the first time in patients between the ages of 30 and 50 years, while gastric ulcer does not usually appear in patients under age 60. These age- and gender-related epidemiological trends reflect three emerging phenomena in peptic ulcer disease: 1) the prevalence of H. pylori infection increases

with age. In developed countries, approximately 10% of the population is infected with H. pylori by age 30 but this figure rises to approximately 60% in persons over age 60 (Peterson, 1991); 2) the use of NSAIDs increases with age; 3) smoking, a risk factor involved in disease development, healing and recurrence, has decreased in younger males but has increased in younger females (Soll, 1993).

In placebo-controlled trials, about 30% of gastric ulcers (Howden and Hunt, 1990), and 40% of duodenal ulcers (Burget et al., 1990), heal spontaneously within four weeks. As with all placebo-controlled studies however, it is hard to predict whether these rates of ulcer healing are due to the natural course of the disease or to psychological factors related to the placebo effect. Obviously both genetic and environmental influences impact upon ulcer healing; however certain exogenous factors stand-out as determinants of the disease's natural course. One of the most important of these is smoking. In both gastric and duodenal ulcer, the number of cigarettes smoked per day correlates with a reduced rate of healing and an increased risk of recurrence. Studies have found that non-smoking is a more positive predictor of both ulcer healing (Sonnenberg et al., 1981) and recurrence (Sontag et al., 1984) than is therapy with H_2-antagonists. NSAID use also reduces the rate of healing, and many patients who develop an ulcer while on NSAID therapy become ulcer-free once the NSAIDs are discontinued. Alcohol consumption in moderate amounts has not been shown to alter healing rates; however, certain beverages such as wine, beer and coffee (both caffeinated and decaffeinated), are strong acid secretagogues and may exacerbate symptoms in some patients.

In general, all of the currently available H_2 receptor blockers have been shown to heal about 70 to 80% of duodenal ulcers after approximately 4 to 6 weeks of therapy, while more

rapid rates of healing are observed following daily omeprazole administration (Soll, 1993). It is clear that the extent and duration of inhibition of gastric acidity correlate with duodenal ulcer healing (Burget et al., 1990). Similar results using antisecretory agents have been obtained for gastric ulcer, although omeprazole therapy does not provide the same rapid rate of healing that it does in duodenal ulcer. Antisecretory agents, by reducing acid ouput and indirectly, pepsin activity, permit endogenous protective mechanisms to repair the damaged mucosa. Sucralfate (McCarthy, 1991) and bismuth (Tytgat, 1987) compounds induce healing in both gastric and duodenal ulcer with efficacies comparable to H_2 receptor blockers; however, their mechanisms of action are not due to reducing or buffering acid in the gut. Exactly how they work remains to be firmly established, but they appear to act primarily by augmenting certain endogenous pro-defensive factors involved in mucosal repair.

Without ongoing maintenance therapy, the majority of patients with healed duodenal and gastric ulcers can expect a recurrence within 6 to 12 months (Wolosin et al., 1989; Sontag, 1988). Before the identification of H. pylori, trends towards a reduction in duodenal ulcer recurrence rates were noted in patients who initially received only bismuth therapy. In these studies, recurrence after twelve months was observed in 55% of patients treated with bismuth, whereas patients who received H_2 blockers had an 85% recurrence rate (Dobrilla et al., 1988). It is now believed that this reduced recurrence rate may be due to the temporary suppression of H. pylori by the antimicrobial actions of bismuth. Due to the high correlation between patients with active NSAID-independent ulcer disease and H. pylori infection, eradication of the bacterium should theoretically produce a rate of zero recurrence. Pooled clinical trials have indicated that following initial antibiotic therapy (which successfully healed

the ulcer), ulcers recurred in more than 80% of patients within one year if eradication was not successful. In those patients whose treatment resulted in eradication, recurrence rates approached 0% (Chiba et al., 1992). Although several therapies have been explored clinically, many involving polypharmacy, no single treatment combination has been shown to eradicate H. pylori beyond approximately 90% efficacy (Anderson, 1994). Currently the most effective therapy for H. pylori infection is a triple therapy combination involving the co-administration of bismuth subsalicylate, metronidazole, and tetracycline or amoxicillin during a two-week period. Unfortunately, due to the complicated nature of the treatment, patient compliance is an important factor in predicating whether or not eradication will be successful (Graham et al., 1992).

The presence of H. pylori and its associated gastritis is now an established risk factor for the development of ulcer disease. It is accepted that H. pylori infection is a causal factor in gastritis. Between 70 and 100% of patients with active chronic gastritis are positive for H. pylori, and the presence or absence of active gastritis correlates with rates of infection or eradication of the bacterium, respectively. The prevalence of H. pylori infection in developed countries is approximately 25 to 50%; however, in developing countries it has been estimated to be as high as 80% (Lambert and Lin, 1994). The higher rate of infection in developing countries is believed to be due to poor sanitation standards, as the bacterium is transmitted by fecal-oral contact. Infected individuals have an estimated lifetime risk of 10 to 20% for the development of peptic ulcer disease, which is a 3 to 4 times greater risk as compared to those who are not infected (Kuipers et al., 1995). Approximately 80 to 100% of patients with duodenal ulcer and 60 to 95% of patients with gastric ulcer are positive for antral H. pylori

infection (Lambert and Lin, 1994). The lower rates of association between gastric ulcer and H. pylori infection are due to the impact of NSAID use as a risk factor for gastric ulcer development. If ulcers associated with NSAID use are eliminated, correlations for gastric ulcer and H. pylori infection are probably similar to those seen with duodenal ulcer (Wyle, 1991).

The use of NSAIDs is a second important factor in ulcer disease etiology, as it is estimated that the prevalence of ulcers in patients using NSAIDs is between 10 and 30% (Heigh, 1994). In the Asprin for Myocardial Infarction Trial, the risk for hospitalization for duodenal ulcer was about ten times greater for subjects who received acetylsalicylic acid compared to those who received placebo (Kurata and Abbey, 1990). NSAIDs can induce lesions by their direct topical effects or their systemic effects. Topical damage is due to the weakly acidic nature of these compounds. In the un-ionized form within the acidic millieu of the stomach, they are able to freely diffuse through the protective mucus layer and penetrate the epithelial cell surface. Once inside the cell they are ionized. Being negatively charged, they are trapped and concentrated within the cell, inducing alterations in cell permeability (Heigh, 1994). Superficial epithelial erosions may result within minutes. Deeper lesions and frank ulcers result from the systemic actions of these agents (Soll, 1993). By inhibiting cyclooxygenase, mucosal prostaglandin synthesis is also inhibited. The result is that all of the protective mechanisms influenced by mucosal prostaglandins, such as bicarbonate and mucus secretion and the maintenance of gastric mucosal blood flow, are reduced. If NSAID therapy is concurrent with administration of the prostaglandin E analogue misoprostol, the incidence of lesions can be reduced (Graham et al., 1988); however the gastrointestinal side-

effects of misoprostol limit its use in some patients.

II) MECHANISMS OF PEPTIC ULCER DISEASE PATHOGENESIS

i) The Interplay of Aggressive and Defensive Factors in Peptic Ulcer Disease

Amongst peptic ulcer patients, no single abnormality stands out as a defining factor in disease etiology. Patients with gastric and duodenal ulcer disease are a heterogenous population, making it difficult to characterize a single set of pathophysiological changes which predict the disease process. Although an imbalance of aggressive and defensive factors within the gut plays the ultimate role in peptic ulcer disease etiology, a primary failure of one or more of these mechanisms is not usually the initiating cause of the disease (Table 2); that is, certain exogenous factors, such as NSAID use or H. pylori infection, must usually be present to precipitate the imbalance. The subsequent disruption of gastrointestinal homeostasis thus favors ulcer development. This occurs via a potentiation of aggressive elements, diminution of the mucosa's intrinsic ability to protect or heal itself in the face of insult, or a combination of both.

Our understanding of the pathogenesis of ulcer disease has undergone several key paradigm shifts in the last 100 years. As scientific knowledge of the control mechanisms surrounding gastric acid secretion grew, so did its role as a factor in ulcer pathogenesis. So established was this belief, that Schwartz proposed the clinical dictum "...without acid gastric juice, no peptic ulcer..." (Schwartz, 1910). In the 1970's, Sir James Black revolutionized gastric ulcer disease therapy when he published findings which related the inhibition of histamine- and pentagastrin-induced gastric acid secretion to blockade of the H_2-receptor

Aggressive Factors

Endogenous:	Exogenous:
Hydrochloric acid	NSAIDs
Pepsin	Ethanol
Gastrin	Caffeine
Leukotrienes	Stress
H. pylori	Cysteamine
Free radicals	Nicotine
↓ GMBF-ischemia	
Ca^{2+}-dependent proteases	
Dysmotility	

Defensive Factors

Endogenous:	Exogenous:
bFGF	Anticholinergics
Sulfhydryls	Antacids
EGF	H_2-antagonists
Mucus	H^+ - pump inhibitors
Bicarbonate	Colloidal Bismuth
Prostaglandins	Sucralfate
EDRF/NO	D_1/DA_1 agonists
SOD/Catalase/GSH	Ca^{++}-antagonists
Gastric Mucosal blood flow	
Polyamines	
Dopamine	
Gangliosides	
IL-1	

Table 2: Aggressive and Defensive Factors Involved in Ulcer Pathogenesis

(Adapted from Glavin and Hall, 1992)

subtype (Black et al., 1972). Because of their clinical efficacy, H_2-antagonists became the therapy of choice for ulcer disease, this despite the fact that most patients with duodenal ulcer are normal to below normal acid secretors. The concept of ulcer disease pathogenesis as a failure of a defensive mechanism was underscored when Isenberg et al. (1987), reported that patients with duodenal ulcer were hyposecretors of duodenal bicarbonate. Basic research and drug development which had so long been focussed on the aggressive factors in peptic ulcer disease, began to explore the pro-defensive aspects of gastrointestinal physiology. With the discovery of H.pylori within the last ten years, it would appear that most of the etiology of peptic ulcer disease has been deduced. This however, does not explain why only a certain proportion of those who are infected with the bacterium develop an outright lesion, making it clear that H.pylori is only a necessary, not a sufficient risk factor, for disease development. The mechanisms by which certain infected individuals are able to resist lesion formation remains to be elucidated.

ii) Control of Gastric Acid Secretion and its Role in Peptic Ulcer Disease

Recognition that gastric acid secretion is a regulated process derived from the work of William Beaumont in the mid-nineteenth century. With his patient Alexis St. Martin, who required a chronic gastric fistula, Beaumont was able to study acid secretion directly. From his observations he concluded that acid secretion was not a continuous process but was instead controlled by a number of factors including emotional state (Beaumont, 1833).

It is now established that there are multiple mechanisms involved in the regulation of gastric acid secretion, including the central and enteric nervous systems, circulating hormones

and paracrine agents. A detailed description of the multiple factors involved in the regulation of acid secretion are beyond the scope of this thesis; however, a brief outline of parietal cell control will be reviewed within the context of peptic ulcer disease.

With the exception of pathological acid hypersecretory states such as those seen in the Zollinger-Ellison syndrome, hypotheses advocating a major role for acid secretion in ulcer disease have been largely dismissed. The presence of a minimum level of acid however, is still a necessary but not sufficient element in the disease process (Soll, 1993). This is evidenced by the efficacy of antisecretory agents in healing lesions. It is very rare for an ulcer to occur in a low acid environment, and a lesion that persists in an achlorhydric state, especially if it is resistant to aggressive antisecretory therapy, is strongly suggestive of malignancy. Determination of acid output in ulcer patients has produced many conflicting results, especially in studies attempting to measure meal-stimulated acid secretion. While basal acid output is elevated in only about one third of duodenal ulcer patients, maximal acid output (the measure of acid output in response to a secretagogue) is increased when compared to normal controls. An increased maximal acid output suggests greater parietal cell mass, and this has been observed in duodenal ulcer patients (Cox, 1952), however it may also indicate altered sensitivity to factors which regulate acid ouput in response to such stimulation. Measurements of meal-stimulated acid output have not been conclusive although duodenal ulcer patients have been shown to have a more sustained acid response following a meal than normal controls (Soll, 1993). This finding, along with the observation that nocturnal acid secretion is elevated in duodenal ulcer, may explain the pattern of symptomatology related to this disease.

Sensory input from the periphery as well as certain emotional factors are able to stimulate acid secretion, implying a central regulatory component. The exact structures within the central nervous system responsible for acid output have not been determined. It is known that the central sensory input from the hypothalamus and visceral sensory input from the nucleus tractus solitarius are integrated within the dorsal motor nucleus of the vagus (DMNV), and that this structure supplies the efferent vagal fibers which innervate the stomach (Hersey and Sachs, 1995). Destruction of the DMNV results in an inhibition of the central component of acid output (Kerr and Preshaw, 1969), while electrical stimulation increases it (Wyrwicka and Garcia, 1979). The vagal efferent fibers from the DMNV do not innervate the parietal cell directly. Instead, the approximately 2,000 vagal fibers synapse on an estimated 10 million ganglion cells within the enteric nervous system, and the post-ganglionic neurons provide the source of acetylcholine which then stimulates the parietal cell (Hoffman and Schnitzlein, 1961). It is not known what role increased vagal tone plays in the alterations in acid output seen in duodenal ulcer patients. Cholinergic input may be hyperactive in a subset of duodenal ulcer patients, as Kirkpartick and Hirschowitz (1980) were able to markedly decrease the elevated basal acid output that is seen in some ulcer patients, by administration of atropine.

Within the acid-secreting oxyntic mucosa, acetylcholine binds to the M_3 muscarinic receptor subtype on the parietal cell. Acetylcholine, via an uncharacterized muscarinic receptor subtype, also binds to nearby enterochromaffin-like cells, where it acts as a stimulus for histamine release. The subepithelial enterochromaffin-like cells comprise one third to one half of the endocrine cells within the oxyntic mucosa and function to synthesize, store and

release histamine which, acting as a paracrine agent, activates H_2 receptors on the nearby parietal cell. In the gastric antrum, gastrin release from the G cells is stimulated by a number of factors, including the chemical content of food in the stomach, gastric distention and central neural pathways. Acting as a hormone within the systemic circulation, gastrin reaches the fundus where it binds to the CCK-B receptor subtype on the enterochromaffin-like cell. Gastrin therefore, stimulates histamine realease from the enterochromaffin-like cells (Prinz et al., 1993).

Administration of the proteolytic enzyme pepsin in combination with acid has been shown to be a more potent ulcerogen than acid alone. Chief cells in the gastric mucosa contain zymogen granules containing the proenzyme pepsinogen. Upon cholinergic stimulation, the chief cells release pepsinogen, which is then cleaved to form active pepsin within the acidic gastric millieu. Pepsins are most active at pH 1.0 to 3.5, and only minimally active up to pH 5.0 (Venables, 1986). In human gastric juice, seven pepsins and one non-pepsin protease have been identified (Etherington and Taylor, 1967). Pepsin 3 is the major form in man, while pepsin 1 accounts for only 3.6% of the total pepsin activity in non-ulcer controls. In patients with gastric and duodenal ulcer, pepsin 1 can account for as much as 23% and 16.5% of the total proteolytic activity respectively (Pearson et al., 1986). In response to histamine, patients with peptic ulcer disease have been shown to secrete pepsin 1 to a significantly greater extent than non-ulcer controls (Taylor, 1970). Pepsin 1 has also been shown to digest gastric mucus at a faster rate when compared to pepsin 3. This difference in mucolytic ability was even more marked as pH increased. At pH 4.0, pepsin 1 had six times the mucolytic activity of pepsin 3. At this same pH, gastric juice obtained from

duodenal ulcer patients exhibited mucolytic activity similar to that seen with pepsin 1 (Pearson et al., 1986). This indicates that the more aggressive form of pepsin may be secreted to a greater extent in ulcer patients, possibly impacting upon ulcer formation or healing.

Given that agents which inhibit acid secretion are successful in promoting ulcer healing at least temporarily, and treatment of especially persistant ulcers has been successful following the creation of an achlorhydric state with high doses of omeprazole, a role for acid in ulcer pathogenesis cannot be entirely eliminated. The most likely explanation is that acid and pepsin activity are opportunistic factors in ulcer formation, taking advantage of insufficient defense and repair mechanisms.

iii) Mucosal Defense Mechanisms and Their Role in Peptic Ulcer Disease

Multiple mechanisms exist to provide for mucosal defense and repair in the presence of any number of aggressive factors. If these endogenous defense mechanisms fail, then epithelial cell damage will occur (Figure 1a). If the mucosa's intrinsic ability to repair itself is also inadequate, then an ulcer will develop (Figure 1b).

In the absence of pathology, the human gastric mucosa is covered by a continuous layer of water-insoluble mucus which varies in thickness from 50 to 450 μm (Allen et al., 1990). This adherent mucus gel forms a physical barrier that protects the underlying mucosal cell layers from the degradative factors contained in the luminal juice. In this capacity its protective role is threefold: 1) it forms a physical barrier against the mechanical forces of digestion; 2) provides a diffusion barrier against pepsin; 3) acts in conjunction with epithelial bicarbonate secretion to form an unstirred layer, buffering acid diffusion (Sellers and Allen,

Exogenous Factors:
Eg. NSAIDs

Acid and Pepsin

Endogenous Factors
Eg. Bile

First Line Defense: Mucus/ Bicarbonate Barrier

Second Line Defense: Epithelial Cell Mechanisms
- Gastric mucosal barrier
- Extrusion of acid

Third Line Defense: Gastric Mucosal Blood Flow
- Removal of back-diffused acid
- Oxygen supply

EPITHELIAL CELL INJURY

Figure 1a: Hierarchy of Mucosal Defense Mechanisms: (The failure of which leads to epithelial cell injury)

First Line Repair: Restitution

Second Line Repair: Cell Replication

ACUTE WOUND FORMATION

Third Line Repair: Angiogenesis/ Remodelling of Basement Membrane

ULCER FORMATION

Figure 1b: Hierarchy of Mucosal Repair Mechanisms: (The failure of which leads to ulcer disease)

(Adapted from Soll, 1993)

1988). Loss of some of this mucus layer is an inevitable result of normal physiological processes. The shear stress of digestion, as well as the presence of proteolytic enzymes such as pepsin within the lumen, are especially detrimental to the integrity of the adherent mucus.

The adherent gel which covers the epithelium is not a homogenous layer of mucus. It is actually an unstirred layer comprised of a variety of substances including water, sloughed epithelial cells, digestive enzymes, nucleic acids and plasma proteins. Approximately 1 to 10% of this mucus secretion by weight is due to the presence of mucin glycoprotein subunits (Sellers and Allen, 1988). These glycoprotein subunits are able to form large polymeric mucin complexes, thus conferring the viscoelastic, gel-forming property to the mucus layer (Allen et al., 1993). A direct correlation exists between the ratio of polymerized mucin to subunit, and the overall strength of the mucus gel. Therefore, it can be predicted that any alteration in the ability of the mucin subunits to polymerize into a stable gel, will alter the protective capability of the adherent layer. Mucus samples obtained from peptic ulcer patients and non-ulcerated controls following surgical resection of the antrum, revealed key differences in the degree of glycoprotein polymerization (Younan et al., 1982). Patients with gastric and duodenal ulcer diseases were shown to have higher levels of a glycoprotein that is of intermediate polymeric structure. Although not reduced to the glycoprotein subunits themselves, this lower molecular weight glycoprotein forms a mucus gel which is structurally less stable than its normal polymeric mucus counterpart. The samples from gastric, duodenal and non-ulcerated controls were enriched with this lower molecular weight glycoprotein by 65%, 50% and 33% respectively. These results imply that a structurally weaker mucus gel covers the mucosa of patients with gastric ulcer disease and to a lesser extent, duodenal ulcer

patients (Younan et al., 1982). These observations however, do not imply that peptic ulcer disease patients have inherent differences in mucus gel structure or that these differences are causally related to lesion formation.

Pepsin is a large molecule and is therefore unable to diffuse through the mucus layer. As mucus is a glycoprotein, it is susceptible to hydrolysis by proteolytic enzymes such as pepsin. Pepsin cleaves the polymeric mucus into its mucin subunits, solublizing the mucus layer and changing its mechanical properties from an "elastic gel" to a "viscous liquid" (Bell et al., 1988). In vivo, the effect of pepsin is to progressively degrade the mucus layer at its luminal surface. Maintenance of an intact mucus gel therefore, is dependent upon new mucus secretion from surface epithelial cells.

The mucus gel layer is able to maintain its mechanical properties following prolonged exposure to a number of mucosal damaging agents including 20 mM sodium taurocholate, HCl at pH 1.0, ethanol (up to 40% v/v) and 2 M NaCl (Bell et al., 1988). Although the mechanical structure of the mucus gel is unaffected by acid in the lumen, hydrogen ions are able to diffuse through the mucus layer. The gel-like quality of the mucus impedes the rate of hydrogen ion diffusion somewhat however, hydrogen ions are still capable of reaching the epithelial surface. Bicarbonate secreted by the surface epithelial cells directly into the mucus gel provides a mechanism for buffering diffusing hydrogen ions.

The adherent mucus functions to restrict bicarbonate diffusion, keeping it concentrated at the mucosal surface and allowing for the neutralization of hydrogen ions within the gel itself. The existence of a gradient of pH 1.0 to 2.0 in the gastric lumen to near neutral at the epithelial surface is evidence that the unstirred layer effectively buffers diffusing

hydrogen ions. Both gastric and duodenal bicarbonate secretion is stimulated by luminal acidity. In animals the duodenal alkaline secretory response to acidity has been found to decrease with age (Kim et al., 1990), an interesting finding given the age-related trends in ulcer disease development in humans. Isenberg et al. (1987) proposed that impaired duodenal bicarbonate secretion may be a factor in the pathogenesis of duodenal ulcer, rather than being secondary to it. In patients with inactive duodenal ulcer, both basal and acid-stimulated bicarbonate secretion from the proximal duodenum was found to be lower when compared to measurements from normal subjects. Resting pH in the duodenal bulb was also lower in duodenal ulcer patients.

Prostaglandins are one of the most potent duodenal bicarbonate secretagogues (Konturek et al., 1983), and in a number of species including humans, the increase in gastric and duodenal bicarbonate secretion induced by luminal acidity is correlated with an increased release of prostaglandin E_2 (Allen et al., 1993). In rats, administration of indomethacin (Takeuchi et al., 1986b), or the phopholipase A_2 inhibitor quinacrine (Takeuchi et al., 1986a), has been shown to inhibit both prostaglandin release and bicarbonate secretion.

If mucosal defense mechanisms such as the mucus/bicarbonate barrier fail and a superficial epithelial lesion results, rapid epithelial restitution will occur within 15 to 30 minutes of an acute insult (Szabo, 1991). In this process, undamaged cells migrate along the basement membrane, covering the site of injury and establishing new cell contacts. Epithelial restitution can occur only if the surrounding microcirculation is maintained and the basement membrane is intact. If blood supply to the mucosa is interrupted and deeper necrosis results, the mucosa must rely on cell proliferation for the repair of damage. As 70-90% of the blood

flow to the stomach reaches the mucosa, maintained gastric mucosal perfusion underlies virtually all gastroprotective mechanisms. The role of gastric microcirculation in mediating its protective abilities is three-fold: 1) it maintains normal acid-base balance through the delivery of bicarbonate from the alkaline tide, or from systemic circulation; 2) it delivers the substrates required for energy-dependent defense mechanisms such as rapid epithelial restitution; and 3) it clears harmful substances such as back-diffused acid from the mucosal interstitium (O'Brien, 1988). The evidence is undeniable that an inverse relationship exists between lesion development and mucosal perfusion. Vasoconstrictive factors such as thromboxane and endothelin produce acute mucosal lesions, whereas administration of agents which augment blood flow are gastroprotective (Allen et al., 1993).

Prostaglandins, a family of long-chain fatty acids formed from arachidonic acid by the enzyme cyclooxygenase, are abundant in the gastrointestinal mucosa. Although the various prostaglandins have different actions on smooth muscle, within the gastrointestinal tract they have been uniformally shown to be protective, especially following oral or topical administration. It was originally believed that prostaglandins reduced mucosal lesion formation by inhibiting acid secretion. However, when administered in anti-secretory doses, prostaglandins still exerted a protective effect, even against a variety of noxious factors including ethanol, HCl, NaOH, and hypertonic NaCl. These results suggest that their 'cytoprotective' action is acid-independent (Robert et al., 1979). The mechanisms by which ethanol induces mucosal damage are not known, although several hypotheses have been proposed (Glavin and Szabo, 1992). Gross histological examination of the stomach after ethanol instillation reveals extensive damage to the mucosal microvasculature that is

suggestive of vascular stasis (Guth et al., 1984). Given that prostaglandin pretreatment is able to prevent ethanol-induced mucosal damage, it was proposed that their mechanism of gastoprotection is due to the maintenance of gastric mucosal blood flow.

Epidemiological data have shown correlations between portal hypertension and gastric and duodenal ulcer disease in man (Mitani et al., 1989). Some studies have linked the two diseases to a decrease in prostaglandin synthesis by the gastric mucosa in patients with cirrhosis (Soll, 1993). However, patients with portal hypertension and portocaval shunting experience a variety of pathophysiological mechanisms which may result in altered gastrointestinal function, including changes in splanchnic circulation and altered hepatic metabolism of gastrointestinal hormones. Propranolol has been successful in lowering portal blood pressure in some patients with portal hypertension. In these patients, the drug has not only been found to prevent variceal hemorrhage but gastrointestinal bleeding as well (Lebrec et al., 1981).

Abnormalities in the gastric mucosa of portal hypertensive rats have been described which increase the mucosa's susceptibility to ethanol-induced damage. Sarfeh et al. (1983), observed that following ethanol instillation into the stomach, portal hypertensive rats developed hemorrhagic lesions of significantly greater severity as compared to sham-operated controls. Similar results were obtained following topical taurocholate administration (Sarfeh et al., 1984). Propranolol was found to significantly reduce both portal pressure and the severity of ethanol-induced lesions in portal hypertensive rats, but not in sham-operated, normotensive controls (Sankary et al., 1986). Cimetidine, at a dose which also lowers portal pressure, also significantly protected portal hypertensive rats against ethanol-induced lesions

(Sankary et al., 1984). As was seen with propranolol, cimetidine did not protect against ethanol-induced mucosal damage in normotensive animals (Robert et al., 1979). However, when propranolol was administered to normotensive mice at higher doses than were used by Sankary et al. (1984), it was also found to be protective against ethanol-induced lesions (Bhandare et al., 1990). Propranolol had previously been shown to increase gastric mucosal blood flow (Lin and Evans, 1973), which may explain some of its gastroprotective capability. Pretreatment with indomethacin blocked the protective effects of propranolol, underscoring the importance of gastric mucosal blood flow and prostaglandin synthesis in mediating gastroprotection.

Altering mucosal hemodynamics may be one mechanism by which clonidine exerts an effect on gastrointestinal function. In this capacity however, studies using clonidine have given conflicting results. Al-Bekairi et al. (1993) reported that clonidine exacerbated mucosal damage in response to ethanol, but that it increased gastric adherent mucus and reduced gastric acid output. Kunchandy et al. (1985) found that clonidine was not protective against ethanol-induced lesions. However, at all doses tested (0.05 to 1.0 mg/kg) clonidine was antisecretory, with maximum inhibition of acid output seen at 0.1 mg/kg. In restraint stress-induced lesions, clonidine (1.0 mg/kg) was also found to be protective. Glavin and Smyth (1995) confirmed the antisecretory potency of clonidine and at doses of 0.01 and 1.0 mg/kg, found it to reduce basal acid output by 37% and 46%, respectively.

Given evidence that clonidine is an I_1-imidazoline agonist (Ernsberger et al.,1987), and that its antihypertensive action may be due to interaction with this receptor, it is important to determine if any of clonidine's effects on gastrointestinal function are mediated by this site.

Bhandare et al. (1991) demonstrated that clonidine has a biphasic effect on ethanol-induced gastric mucosal damage. At a maximally protective dose of 0.1 mg/kg, clonidine reduced the area of hemorrhagic lesions, an effect that was prevented by yohimbine pretreatment. At 1.0 mg/kg, clonidine exacerbated mucosal injury, while yohimbine pretreatment had no effect. Oxymetazoline, another imidazoline compound, and α_2-agonist, also exacerbated injury at higher doses, while α-methyldopa (a catecholamine) and guanabenz (a guanidine) were both protective. These data therefore, suggest a role for the imidazoline receptor in gastrointestinal function (see below).

III) IMIDAZOLINE RECEPTOR PHARMACOLOGY

Until very recently, the antihypertensive action of clonidine was believed to be due to activation of α_2-adrenoceptors within the rostral ventrolateral medulla (Bousquet et al., 1981). This brainstem pressor region has been implicated in the regulation of sympathetic vasomotor tone, and in the integration of most sympathetic vasomotor reflexes (Granata et al., 1986). One of clonidine's major side-effects, and one which limits its use in the treatment of hypertension, is its sedative property. Clonidine-induced sedation is believed to originate within the locus coeruleus, and results from the reduction in sympathetic outflow that accompanies α_2-adrenoceptor activation. In 1984 however, a substance was partially purified from the bovine brain which displaced clonidine binding to α_2-adrenoceptors in the rat brain (Atlas and Burstein, 1984). This substance was termed clonidine-displacing substance and as a result of its discovery new vistas in pharmacological research emerged. In addition to its affinity for the α_2-adrenergic site, clonidine-displacing substance was also shown to bind to the nonadrenergic imidazoline receptor. This novel class of receptor recognizes ligands

which possess an imidazoline ring or related moiety as part of their molecular structure. Clonidine, an imidazolidine, contains such a ring and has been shown to bind to the imidazoline receptor (Ernsberger et al., 1987). It is now believed that clonidine's antihypertensive action is actually mediated through the imidazoline receptor, which is found in high concentrations within the rostral ventrolateral medulla. A new generation of antihypertensive agents more specific for the imidazoline receptor than clonidine, is currently in clinical use in some European countries. One of these compounds, moxonidine, lowers blood pressure with comparable efficacy as compared to clonidine but exhibits fewer sedative side-effects (Chrisp and Faulds, 1992). This absence of concomitant sedation may be due to the fact that imidazoline receptors are absent from the locus coeruleus (Ernsberger and Collins, 1993).

Although it is established that clonidine binds to α_2 receptors, early experimental findings in this field of pharmacology raised some doubt as to the true nature of its receptor specificity, especially in terms of mediating its physiological effect upon blood pressure. Unexpected experimental observations using a number of structurally-related compounds further stimulated debate. For example, in competition binding studies using tissue from the ventrolateral medulla, norepinephrine displaced the binding of the α_2-agonist [^{3}H]*p*-aminoclonidine to a maximum of 70%. The remaining 30% of the binding of [^{3}H] *p*-aminoclonidine was insensitive to norepinephrine, but displayed high affinity for imidazole compounds. Some of the same ligands which inhibited [^{3}H]*p*-aminoclonidine binding in the ventrolateral medulla had no effect in cortical tissue, indicating a regional distribution for the non-adrenergic, imidazole-preferring site (Ernsberger et al., 1987). Following injection into

brainstem ventricles, the α-antagonist phentolamine was found to paradoxically reduce blood pressure, an effect which was potentiated by clonidine co-administration (Bogaievsky et al., 1974). The involvement of the α_2 receptor as sole mediator of this phenomenon could not be explained, as both agents lowered blood pressure by reducing sympathetic outflow. Similarly, the H_2-antagonist cimetidine has been shown to lower blood pressure and heart rate following direct administration into the rostral ventrolateral medulla (Karppanen, 1981). Therefore, a seemingly diverse group of ligands are able to produce a similar clonidine-like antihypertensive response. Examination of the molecular structures of these compounds revealed certain similarites: clonidine, the prototypical α_2-agonist, is an imidazolidine; phentolamine, an imidazoline; and cimetidine, an imidazole. Although not related functionally, it was hypothesized that these agents were giving rise to similar physiological responses because of their interaction with an "imidazoline-preferring receptor site".

Seminal research into these observations was conducted by Bousquet et al. (1984), who hypothesized a hypotensive action similar to that of clonidine, following microinjection of the highly selective α_2-agonist α-methylnorepinephrine into the nucleus reticularis lateralis of the ventral medulla, a tonic vasopressive structure and important site for clonidine's pharmacological action. They further speculated that agonists with the opposite selectivity (ie. α_1-agonist) microinjected into the same brainstem region would not be expected to have the same effect. After administration of the catecholamine α-methylnorepinephrine, no changes in blood pressure were observed. Similar results were observed following norepinephrine administration into the same region (Bousquet and Schwartz, 1983). In contrast, the α_1-agonists cirazoline and ST 587, both imidazolines, reduced blood pressure

in a dose dependent fashion (Bousquet et al., 1984). Differential effects between these two structurally diverse compounds are observed within the nucleus tractus solitarius. However in this region, the catecholamines are hypotensive and the imidazolines have no effect (De Jong and Nijkamp, 1976).

A variety of terms have been used to describe the receptive site for imidazoline agents including imidazoline-preferring receptors, nonadrenergic idazoxan binding sites and imidazole guanidinium receptor sites. However, the term imidazoline receptor, of which there exists I_1 and I_2 subtypes, is now standardized nomenclature. Radioligand binding studies have since confirmed that clonidine binds with equal affinity to both α_2- and I_1 receptors within the rostral ventrolateral medulla (Ernsberger et al., 1987). That the imidazoline receptor is distinct from adrenergic sites is evidenced in part by the observation that imidazoline receptors do not bind phenylethylamines, those prototypical α-agonists which include the endogenous catecholamines norepinephrine and epinephrine (Ernsberger et al., 1995). Various other compounds of the imidazoline class including rilmenidine, bind to I_1 receptors with varying affinities as compared to the α_2 site. Although no extremely I_1 specific compounds have been identified to date, moxonidine has been shown to be the most specific I_1 receptor agonist (Ernsberger et al., 1993) while efaroxan has been shown to be the most specific antagonist (Ernsberger et al., 1992).

In radioligand binding studies using [^{3}H]clonidine, [^{3}H]*p*-aminoclonidine and [^{125}I]*p*-iodoclonidine, the distribution of I_1-receptors has to a large extent, been found to parallel the distribution of α_2-receptors. Both of these receptor types have been observed in the rostral ventrolateral medulla (Ernsberger et al., 1993), carotid body, and proximal and distal tubule

of the nephron (Ernsberger et al., 1995). The ratio of the density of I_1-receptors as compared to α_2-receptors however, varies depending on the tissue assayed. I_1 sites are not labelled on astrocytes, and I_1 sites are outnumbered by α_2 sites by a 10:1 ratio in cerebral cortex. Conversely, adrenal chromaffin cells express the I_1-receptor in the absence of the α_2-receptor. Although these two receptor types exist together in many tissues, the fact that one type can be expressed in the absence of another makes it unlikely that the imidazoline site is simply a subunit of the α_2-receptor. In tissues assayed to date, in comparison to I_1 receptors, I_2 sites are much more common and widely distributed. I_2 receptors are expressed in kidney, liver, brain, cultured astrocytes, adipocytes, urethra, platelets, pancreatic islets cells and adrenal chromaffin cells (Reis et al., 1995). The subcellular distribution of the two receptors is unequal as I_1 sites are localized to the plasma membrane whereas I_2 sites are localized to the mitochondrial membrane.

Little is known about the mechanisms by which imidazoline receptors transduce their signals upon ligand binding. Studies however, have reported that it is not through the generation of cyclic nucleotides or activation of phospholipase C (Regunathan et al., 1991). Imidazoline receptor signal transduction pathways may be via the activation of phospholipase A_2 as moxonidine is able to stimulate the release of prostaglandin E_2 from I_1-imidazoline receptor enriched rat pheochromocytoma cells (Ernsberger et al., 1995). Interestingly, cimetidine also elicited a release of prostaglandin E_2, an effect which is consistent with its profile as an I_1-imidazoline receptor agonist (Ernsberger et al., 1990). The implications for a phospholipase A_2 mediated signal transduction pathway with its associated release of prostaglandins on gastrointestinal function is therefore, of great interest.

When Atlas and Burstein began their series of investigations which would eventually lead to the partial purification of clonidine-displacing substance, they did so without knowledge of the existence of an imidazoline receptor (Atlas and Burstein, 1984). They proposed the existence of a second endogenous substance which, in addition to norepinephrine, could bind to the α_2-receptor and (possibly) regulate blood pressure (Atlas and Burstein, 1984). Using a four step procedure (methanolic extraction, ion-exchange chromatography, zone electrophoresis, and high pressure liquid chromatography (HPLC)) they isolated a substance from the bovine brain with an estimated molecular mass of 500 ± 50 Da. The novel non-catecholamine substance was able to displace [^{3}H]clonidine binding to α_2-adrenoceptors in rat brain membranes, but had no effect on binding at either α_1 - or β-adrenergic receptors. Clonidine-displacing substance as purified by Atlas and Burstein is a bioactive compound. When microinjected into the nucleus reticularis lateralis it produced hypertension, inducing an increase in mean arterial pressure without a significant alteration in heart rate. Following disruption of the blood brain barrier with mannitol, a significant increase in mean arterial pressure accompanied injection of clonidine-displacing substance into the vertebral artery. Pretreatment with clonidine-displacing substance prevented the hypotensive and bradycardic effects of clonidine administration into the vertebral artery (Bousquet et al., 1987). Since microinjection of both clonidine and clonidine-displacing substance into the nucleus reticularis lateralis effects blood pressure (although in opposite directions), Bousquet et al. (1987) proposed that clonidine-displacing substance might be an endogenous non-catecholamine ligand of the imidazoline receptor. Following the results of binding assays that assessed the affinity of clonidine-displacing substance for imidazoline and

α_2-adrenergic receptors, Ernsberger et al. (1988), supported this view. Clonidine-displacing substance competitively inhibited [^{3}H]*p*-aminoclonidine binding within the rostral ventrolateral medulla at both imidazoline and α_2-adrenergic receptors; however, it displayed a 30 times greater selectivity for the imidazoline binding site as compared to the α_2 receptor. Although clonidine-displacing substance competitively displaces clonidine in binding assays and has been shown to block clonidine's hypotensive actions *in vivo*, the nature of clonidine-displacing substance's interaction with the imidazoline receptor is not known.

Because their assay yielded only a small amount of clonidine-displacing substance, the compound's molecular structure could not be identified. Recently, Li et al. (1994) attempted to determine its structure using extracts from bovine brain. HPLC analysis revealed a retention time identical to that of agmatine, the 130 Da decarboxylation product of l-arginine. Mass spectroscopy confirmed that the purified compound was actually agmatine. Previously thought to serve only as a precursor to polyamine synthesis in lower life forms such as bacteria, plants and some invertebrate species, this was the first demonstration of agmatine's presence in mammalian tissue. In radioligand binding studies using bovine cerebral cortical tissue, agmatine was shown to displace [^{3}H]*p*-aminoclonidine from α_2-adrenoceptors. Within the ventrolateral medulla, in the presence of 10 μM epinephrine to obviate any binding effects at α_2-receptors, agmatine displaced [^{3}H]*p*-aminoclonidine from I_1-receptors. IC_{50} values were lower for binding at the I_1 sites as compared to the α_2 sites indicating a higher affinity of agmatine for the imidazoline receptor. Within bovine adrenal chromaffin cells, agmatine displaced [^{3}H]idazoxan to I_2-receptors, and agmatine was able to cause a dose-dependent release in catecholamines. They concluded that agmatine was one clonidine-displacing

substance and an endogenous ligand for the imidazoline receptor. Piletz et al. (1995), reported a biphasic effect of agmatine binding at the I_1-imidazoline receptor indicating high and low binding affinities for the ligand. Agmatine however, has been shown to have a selectivity for the high affinity I_1-imidazoline receptor which is at least 200 times greater than that for α_2-adrenergic sites.

Agmatine's distribution has been assayed in the rat and was found to be widely distributed. Concentrations of the amine were found to be highest in the stomach; however, levels of the amine were also high in the aorta and to a lesser extent, kidney, heart, liver and brain (Raasch et al., 1995). Arginine decarboxylase, the enzyme responsible for the synthesis of agmatine has been found in the rat brain, providing evidence that agmatine is an endogenously produced substance in mammals, instead of being a byproduct of enteric bacterial metabolism or absorbed from the diet. Arginine decarboxylase is almost exclusively associated with the mitochondrial membrane (Li et al., 1995), an important observation in view of the findings that I_2 receptors have also been localized to the mitochondrial membrane. Li et al. (1995), proposed that l-arginine enters the cell via facilitated transport and is converted to agmatine on the mitochondrial membrane. Agmatine is then able to bind to the nearby I_2 receptors, or diffuse through the cytoplasm to the cell membrane where it can bind to either α_2 or I_1 receptors. As plasma concentrations of agmatine in the rat are substantial, it is speculated that agmatine can also leave the cell where it was synthesized and enter circulation.

Moxonidine is an antihypertensive agent that has been shown to exhibit fewer anti-salivary and sedative side-effects as compared to clonidine (Chrisp and Faulds, 1992). It is

a highly selective I_1-imidazoline agonist, and depending on the tissue assayed, binds to the I_1-imidazoline receptor with 40-700 times greater specificity as compared to the α_2-receptor (Ernsberger et al, 1993). It is now believed that the antihypertensive actions of moxonidine are mediated by imidazoline receptors in the nucleus reticularis lateralis (Ernsberger et al., 1993). Glavin and Smyth (1995) observed that moxonidine exerts profound effects on gastrointestinal function. Moxonidine was found to be protective against ethanol-induced gastric mucosal lesions and in pylorus-ligated rats, it significantly reduced both pepsin and acid output (0.1 and 1.0 mg/kg doses). On measures of acid output in conscious rats, moxonidine administered intraperitoneally (ip) was significantly antisecretory, with maximum inhibition of acid output equal to 100% at the 1.0 mg/kg dose and a calculated $ED_{50} \approx 0.04$ mg/kg ip. This was a far greater inhibition than was seen following clonidine administration. More significantly, moxonidine also raised intragastric pH, suggesting a possible role in bicarbonate secretion.

Given that peripherally administered moxonidine is antisecretory, we proposed to give the drug centrally by administration directly into the lateral cerebral ventricle (icv). Our goal therefore, was to determine whether or not moxonidine, acting at central I_1-imidazoline receptors, would alter gastric acid output in a fashion similar to that observed following its peripheral administration. In order to determine the relative contributions of peripheral and central I_1-imidazoline receptors in mediating acid secretion, subsequent experiments would then involve pretreating the animals with the I_1-imidazoline receptor antagonist efaroxan (ip or icv) prior to giving moxonidine (ip or icv). Moxonidine has previously been shown to decrease both acid and pepsin output (two aggressive factors involved in ulcer disease

pathogenesis). We therefore, proposed to test this compound in animals exposed to cold restraint stress, to determine if it would augment a defensive factor (gastric adherent mucus) involved in gastric mucosal protection. Given that moxonidine is generally associated with gastroprotection, we also speculated that its protective actions might be extended to indomethacin-induced antral lesions. As agmatine is a putative endogenous ligand of the imidazoline receptor (Li et al., 1994), we hypothesized that, after testing it in each of our experimental protocols, its gastrointestinal effects would parallel those of moxonidine. Finally, given that agmatine levels have been assayed in the rat and found to be in highest concentrations in the stomach (Raasch et al., 1995), we administered difluoromethylarginine (DFMA) an inhibitor of arginine decarboxylase. We hypothesized that inhibiting agmatine synthesis would bring about changes in gastric acid secretion which are opposite to those seen following exogenous agmatine administration.

MATERIALS AND METHODS

Animals and Drugs

Male Sprague-Dawley rats were used in all experiments. Animals undergoing successive gastric acid secretion collections weighed 160 to 180 g at the time of gastric cannula implantation. Animals for restraint stress and pylorus ligation protocols were 290 to 210 g, and those used for indomethacin-induced antral lesion experiments weighed 290 to 310g. Animals were housed in a humidity-, temperature-, and light-controlled environment with food and water available *ad libitum.* Where required, animals were anesthetized with

sodium pentobarbital 65.0 mg/kg ip (MTC Pharmaceuticals, Cambridge, ON) and chloral hydrate 300.0 mg/kg ip (BDH, Toronto, ON) or ether (Mallinckrodt, Paris, KN). Moxonidine HCl was a gift from Beiersdorf AG, Hamburg, Germany. Agmatine and indomethacin were purchased from Sigma (St. Louis, MO). Efaroxan was purchased from Research Biochemicals International (Natick, MA). DFMA was a gift from Marion Merrell Dow (Cincinnati, OH). All drugs were dissolved in normal saline with the exception of indomethacin, which was dissolved in a solution of 60% ethanol to 40% saline.

Gastric Cannulae Surgical Preparation

Ongoing measurement of gastric acid secretion required that animals be implanted with chronic indwelling gastric cannulae according to the method of Pare et al., (1977). The spool-shaped stainless steel cannulae measured 12 mm long, with outer flanges equalling 12 mm in diameter. A 1 cm^2 piece of monofilament knitted polypropylene mesh (Marlex, Bard Canada Inc., Mississauga, ON) was attached mid-way down the cannula barrel with acrylic dental cement (Lang's Jet Acrylic, type 2, class 1). Under sodium pentobarbital and chloral hydrate anesthesia, a laparotomy was performed. The incision, 1-2 cm lateral to midline and 1-2 cm caudal to sternum, allowed for stomach access, with care being taken not to damage the vagus nerve. The inner flange of the cannula was inserted into the rumen just above the transverse ridge, thereby avoiding injury to the secretory cells of the glandular stomach. The mesh lay adjacent to the outside of the stomach where it served as a site for adhesion development between the stomach and abdominal muscle layers. This kept the cannula in place post-operatively. A series of purse-string stitches (2-0 silk) around the cannula

prevented the stomach contents from leaking into the peritoneal cavity. A second, smaller incision was made at midline and the cannula pulled through this second incision allowing for externalization of the outer flange of the cannula. The main incision was closed with gut suture and 2-0 silk. Following a ten day recovery period, secretory testing began.

ICV Cannulae Implantation

In those experiments requiring drug administration directly into the lateral cerebral ventricle, animals were implanted with an icv cannula according to the method of Hall et al., (1993). Using this method, 99 to 100% of the animals completed their respective experimental series' without cannula problems. Ten days following abdominal surgery, animals were anesthetized with sodium pentobarbital and chloral hydrate and placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). A 1 cm^2 piece of scalp and the underlying periosteum were removed, and blood vessels cauterized to allow for a dry bone surface. Coordinates for cannula placement were 0.3 mm posterior to bregma, and 1.4 mm lateral to midline (Paxinos and Watson, 1982), and a hole was drilled through the skull. Three additional holes were then drilled in a triangular pattern (one rostral, two caudal) along the perimeter of the exposed bone, and stainless steel bone screws threaded into each hole. A piece of 1 cm^2 monofilament polypropylene mesh (Marlex, Bard Canada Inc, Mississauga, ON) was fitted over these screws and sutured to the scalp using 2-0 silk. The cannula was then sunk to a depth of 3.5 mm below the surface of the skull (Paxinos and Watson, 1982). Acrylic dental cement (Lang's Jet Acrylic, type 2, class 1) was built-up around the cannula for stability. After a three day recovery period secretory testing began.

Gastric Acid Secretion

At 0900 h on the day of secretory testing, the abdominal cannulae were rinsed with distilled water to remove residual food particles. Cannulae were allowed to drain for 20 min to prevent water contamination of the secretion sample. A 12 cm stainless steel catheter was attached to the gut cannula. The other end of the catheter was attached to a collection vial. A secretory collection session occurred over three hours and consisted of the following: a one hour pre-injection baseline control collection followed by drug or vehicle administration (either ip or icv); a second one hour collection; and a final one hour collection which served as a post-injection 'control'. All secretion experiments began and ended with vehicle administration. Therefore, all animals received an initial vehicle, all drug doses and a final vehicle, and each animal served as its own control. Secretion collection sessions were separated by 72 hr to prevent dehydration and to allow for drug washout. In experiments requiring pretreatment, 30 min separated successive drug administrations.

Where required, ip injections consisted of the following: saline vehicle, moxonidine (1.0 mg/kg see Glavin and Smyth, 1995), efaroxan (0.1, 0.5, 1.0, 5.0, 10.0, or 20.0 mg/kg), agmatine (0.1, 0.5, 1.0, 5.0, 10.0, or 20.0 mg/kg), or DFMA (10.0, 20.0 or 40.0 mg/kg) in a volume of 1 ml/kg. Where required, icv injections consisted of the following: saline vehicle, moxonidine (0.01, 0.1, 1.0, or 5.0 μg), efaroxan (1.0, 5.0, 10.0 or 20.0 μg), agmatine (0.5, 1.0, or 2.5 μg), or DFMA (0.01, 0.1, 1.0, or 2.0 μg) in a volume of 5.0 μl over a one-minute period.

The secretion samples collected were centrifuged at 3000 x g for 10 min to remove debris, and a sample titrated to pH 7.0 with 0.01M NaOH (Mettler DL-21 autotitrater) for

determination of acid output. Acid output is expressed as mmol/hour.

Stress-induced Gastric Mucosal Injury

Animals were deprived of food but not water for 24 hours. They received vehicle (distilled water) or agmatine (0.1, 1.0, 10.0, or 20.0 mg/kg ip), and were immediately restrained in a cold room (4-6 °C) for three hours (Glavin et al, 1994). After sacrifice by cervical dislocation, their stomachs were removed, opened along the greater curvature, and rinsed with distilled water. Following fixation with 10% (v/v) formalin, the severity of gastric mucosal injury was assessed using a dissecting microscope, by an observer blinded to the treatment conditions. The length of lesions was cumulated and results are expressed in millimeters.

Gastric Adherent Mucus

Animals were deprived of food but not water for 24 hours, and housed in wire cages to prevent coprophagia. Where required, they received intraperitoneal injections of saline vehicle, moxonidine (0.01, 0.1, or 1.0 mg/kg), efaroxan (20.0 mg/kg), or agmatine (0.1, 1.0, 10.0, or 20.0 mg/kg). Pretreatment experiments required injections be separated by 30 min. The animals were then restrained in a cold room (4-6°C) for three hours as previously described (Glavin et al., 1994). Following exposure to the restraint stress, the animals were killed by cervical dislocation and their glandular stomachs removed, weighed and assayed for gastric adherent mucus concentration via the method of Kitigawa (Kitigawa et al., 1986). Results are expressed as μg/g glandular tissue.

Pylorus Ligation

Animals were deprived of food but not water for 24 hours. Under ether anesthesia a midline laparotomy was performed to access the pylorus, which was then ligated with 2-0 silk with care taken to avoid trauma to the vagi (Shay et al., 1945). Immediately following surgery rats received an intraperitoneal injection of either vehicle (distilled water) or agmatine (0.1, 1.0, 10.0, or 20.0 mg/kg). Four hours later the animals were sacrificed by cervical dislocation and their stomachs removed. Secretion volume was recorded (expressed in ml). Aliquots of stomach contents were centrifuged at 3000 x g for 10 min, and the supernatant then titrated to pH 7.0 with 0.01 M NaOH (Mettler DL-21 autotitrator). Results are expressed as mmol/hour. A second sample of gastric contensts was taken for the determination of pepsin secretion according to the method of Dupuy and Szabo (1986). Results are expressed as total pepsin output (mg/4 hr) and pepsin concentration (mg/ml).

Indomethacin-induced Antral Lesions

Animals were deprived of food but not water for 24 hours, and housed in wire-cages to prevent coprophagia. Antral lesion induction was via the method of Kuratani et al., (1992). At the end of the fasting period, animals were refed standard laboratory rat chow for 1 hr. The animals were again deprived of food, and immediately received indomethacin (32.0 mg/kg sc), and either saline vehicle or moxonidine (0.01, 0.5 or 1.0 mg/kg ip). Six hours later they were sacrificed by cervical dislocation. The stomachs were removed, opened along the greater curvature, rinsed with distilled water, and fixed with 10 % (v/v) buffered formalin. Examination and quantification of ulcer severity were performed using a dissecting

microscope, by an observer blinded to the treatment conditions. Results are expressed in millimeters.

Data Analysis

Data from gastric acid secretion experiments were analyzed by a repeated-measures ANOVA followed by a Tukey HSD test. Stress-induced mucosal lesion, gastric adherent mucus, pylorus ligation and indomethacin-induced antral lesion data were analyzed by factorial ANOVA followed by a Tukey HSD. Significance was considered at $p< 0.05$.

RESULTS

Following icv administration, moxonidine reduced basal gastric acid output. The results were significant at all doses tested (0.01, 0.1, 1.0 and 5.0 μg), with percent inhibition of acid secretion equal to 24, 21, 34, and 38%, respectively ($p<0.05$) (Figure 2). Preliminary data show that this antisecretory effect of icv moxonidine was not antagonized by pretreatment with a 20.0 μg icv dose of efaroxan (data not shown). Pretreatment with efaroxan icv at doses of 10.0 or 20.0 μg was not able to fully block the antisecretory effect of peripherally administered moxonidine at a dose of 1.0 mg/kg (Table 3). Pretreatment with peripheral efaroxan at doses of 10.0 or 20.0 mg/kg ip failed to block the reduction in acid output which was seen following centrally administered moxonidine at doses of 0.1 and 1.0 μg icv (Table 3). This latter combination (efaroxan ip and moxonidine icv) appeared to actually potentiate the antisecretory effects of moxonidine.

Moxonidine's ability to reduce basal acid output was antagonized following peripheral administration of both efaroxan and moxonidine. (Table 4). Pretreatment with efaroxan at doses of 20.0, 10.0 and 5.0 mg/kg ip completely blocked the antisecretory effects of a 1.0 mg/kg ip dose of moxonidine. The ability of moxonidine to reduce basal acid output began to break through only after the dose of efaroxan was reduced to 1.0 mg/kg ip. Efaroxan at doses of 0.5 and 0.1 mg/kg ip did not block, but instead appeared to potentiate, moxonidine's antisecretory actions.

When administered either centrally at doses of 1.0, 5.0 10.0, or 20.0 μg icv (Figure 3) or peripherally at doses of 1.0, 5.0, or 10.0 mg/kg ip (Figure 4), efaroxan alone did not

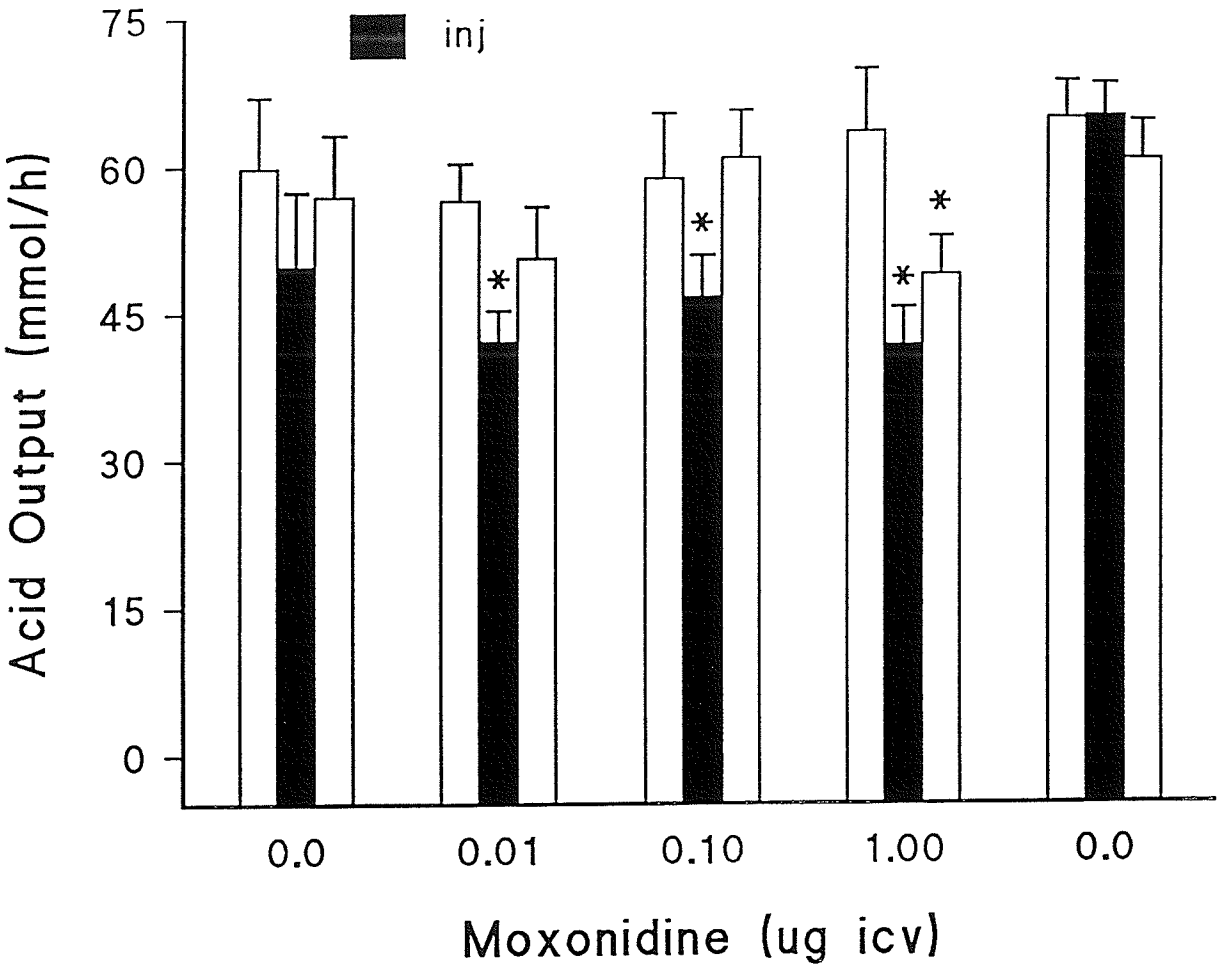

Figure 2: Effect of Centrally Administered Moxonidine on Gastric Acid Secretion in Conscious Rats

Rats (n = 6-8) were infused with either saline vehicle or moxonidine in a 5 μl volume over a 1-min period at the beginning of the second hour of collection (solid bars). Open bars represent pre- and post-injection baseline collections of gastric acid during which no injections occurred. Results are expressed as mmol acid/ hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus preinjection baseline.

Route of Administration: icv	Route of Administration: ip	% Inhibition of Acid Secretion
Vehicle	Vehicle	0
Moxonidine 0.1 μg	Vehicle	21
Moxonidine 1.0 μg	Vehicle	34
Vehicle	Moxonidine 1.0 mg/kg	65[see note]
Efaroxan 10.0 μg	Moxonidine 1.0 mg/kg	46
Efaroxan 20.0 μg	Moxonidine 1.0 mg/kg	48
Moxonidine 0.1 μg	Efaroxan 10.0 mg/kg	31
Moxonidine 0.1 μg	Efaroxan 20.0 mg/kg	33
Moxonidine 1.0 μg	Efaroxan 10.0 mg/kg	65
Moxonidine 1.0 μg	Efaroxan 20.0 mg/kg	68

Note: Data from Glavin and Smyth (1995).

Table 3: Effect of Efaroxan Pretreatment on the Antisecretory Effect of Moxonidine

In all experiments efaroxan pretreatment occurred 30 min prior to moxonidine administration. All animals (n = 6-8 per group) initially received saline vehicle which served as a control. A first group of animals was given efaroxan icv at doses of 10.0 and 20.0 μg, followed by moxonidine ip (1.0 mg/kg). A second group of animals received efaroxan ip (10.0 and 20.0 mg/kg) followed by moxonidine icv (1.0 μg). Results are expressed as % inhibition of acid output as compared to saline vehicle controls.

Route of Administration: ip	Route of Administration: ip	% Inhibition of Acid Secretion
Vehicle	Vehicle	O
Vehicle	Moxonidine 1.0 mg/kg	66
Efaroxan 20.0 mg/kg	Moxonidine 1.0 mg/kg	0
Efaroxan 10.0 mg/kg	Moxonidine 1.0 mg/kg	0
Efaroxan 5.0 mg/kg	Moxonidine 1.0 mg/kg	0
Efaroxan 1.0 mg/kg	Moxonidine 1.0 mg/kg	7
Efaroxan 0.5 mg/kg	Moxonidine 1.0 mg/kg	84
Efaroxan 0.1 mg/kg	Moxonidine 1.0 mg/kg	85

Table 4: Effect of Peripherally Administered Efaroxan Pretreatment on the Antisecretory Effect of Peripherally Administered Moxonidine

Animals (n=6-8) initially received saline vehicle which served as control. Pretreatment with efaroxan (0.1, 0.5, 1.0, 5.0, 10.0 and 20.0 mg/kg ip) occurred 30 min prior to moxonidine administration (1.0 mg/kg ip). Results are expressed as % inhibition of acid secretion relative to saline vehicle.

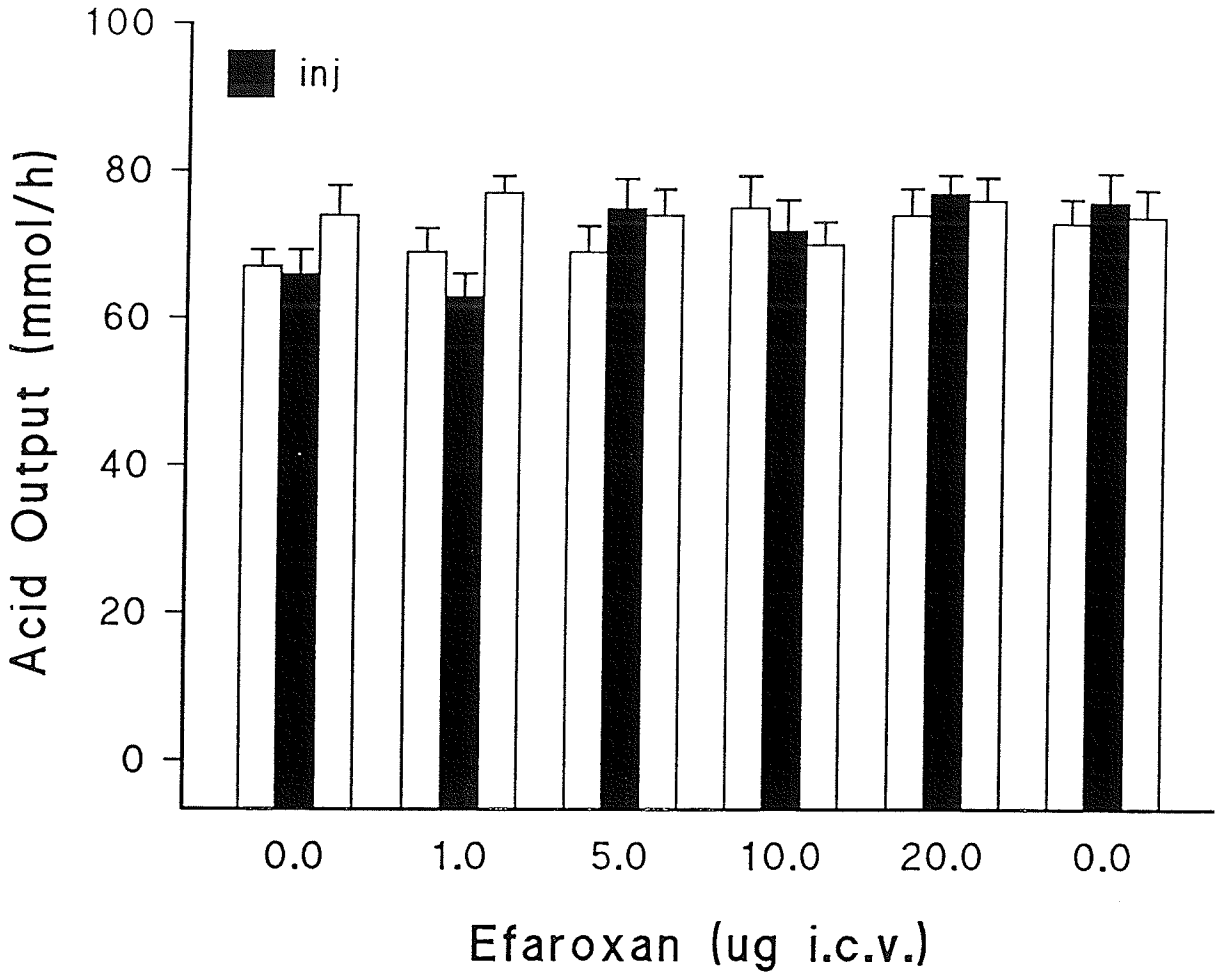

Figure 3: The Effect of Centrally Administered Efaroxan on Gastric Acid Secretion in the Consious Rat

Rats (n=6-8) were infused with either saline vehicle or efaroxan in a 5-μl volume over a 1-min period at the beginning of the second hour of collection (solid bars). Open bars represent pre- and post-injection baseline collections of gastric acid during which no injections occurred. Results are expressed as mmol acid/ hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus pre-injection baseline.

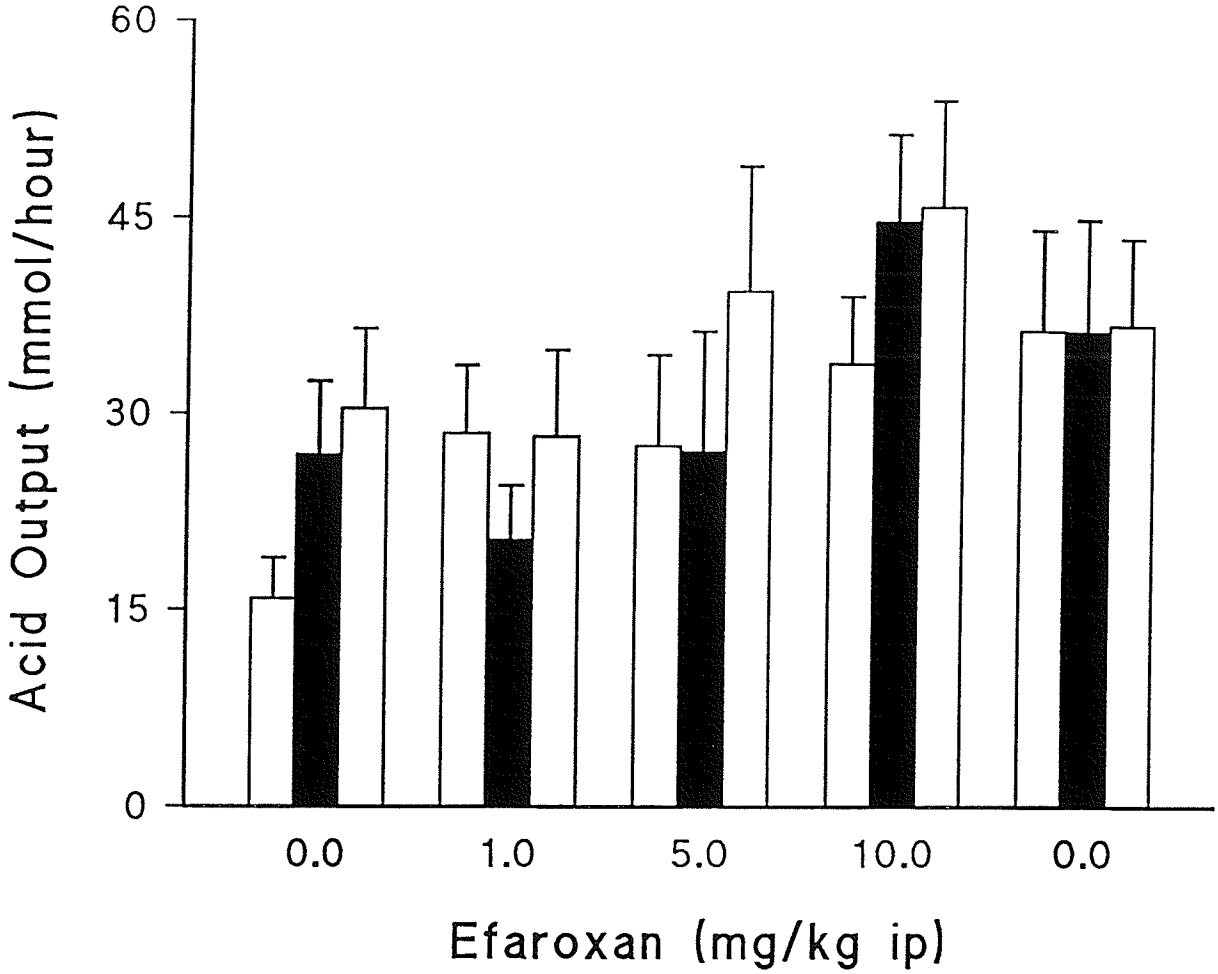

Figure 4: Effect of Peripherally Administered Efaroxan on Gastric Acid Secretion in the Conscious Rat

Animals (n=6-8) were given individual injections of saline vehicle, efaroxan at doses of 1.0, 5.0 and 10.0 mg/kg ip, and vehicle again. Each injection was separated by 72 hours. Pre- and post-injection baseline collections of gastric secretion are represented by the open bars. Solid bars indicate 1 hour collection immediately following injection. Results are expressed as mmol acid/ hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus pre-injection baseline.

significantly alter basal acid output as compared to pre-injection baseline controls. Similarly, no significant changes in acid output were seen following administration of DFMA either centrally at doses of 0.01, 0.1, 1.0, or 2.0 μg icv (Figure 5), or peripherally at doses of 10.0, 20.0 or 40.0 mg/kg ip (Figure 6).

Centrally administered agmatine at the two highest doses tested, significantly increased basal acid output. This effect was manifest especially during the second hour following drug administration, with acid output augmented by 31 and 44%, at doses of 1.0 and 2.5 μg icv, respectively ($p< 0.05$) (Figure 7). Peripherally administered agmatine significantly increased acid output at all doses tested ($p< 0.05$) (Figure 8). Maximum augmentation of acid output equalled 140%, as compared to pre-injection baseline controls, and occurred during the second hour following drug administration at a dose of 10.0 mg/kg ip. Pretreatment with efaroxan at doses 1.0 and 5.0 mg/kg ip blocked the increase in acid output seen following a 10.0 mg/kg ip dose of agmatine (Figure 9). This antagonism was observed with doses of efaroxan similar to those previously found to block moxonidine's antisecretory action. The prosecretory effect of agmatine was not antagonized by efaroxan at doses of 0.1 or 0.5 mg/kg ip.

In the pylorus ligation model, agmatine significantly increased gastric acid secretion at doses of 1.0, 10.0 and 20.0 mg/kg ip ($p< 0.05$). At all doses tested, agmatine ip. significantly increased both pepsin output as well as pepsin concentration ($p< 0.05$). (Table 5).

In animals exposed to cold-restraint stress, agmatine at doses of 1.0 and 20.0 mg/kg ip, significantly increased the severity of gastric mucosal lesions. (Figure 10). Agmatine at

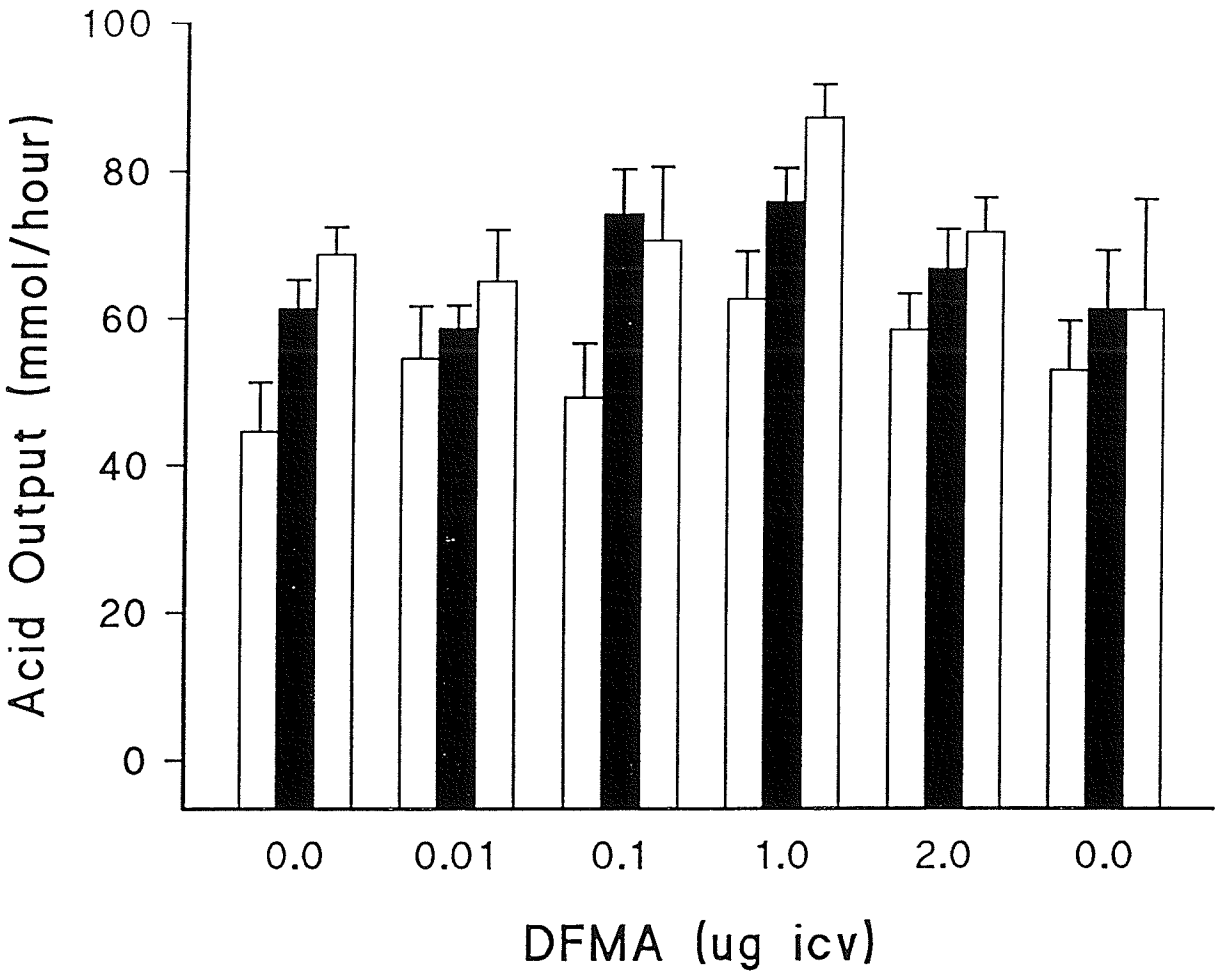

Figure 5: Effect of Centrally Administered DFMA on Gastric Acid Secretion in the Conscious Rat

Animals (n=6-8) were given individual injections of saline vehicle, DFMA at doses of 0.01, 0.1, 1.0 and 2.0 μg icv, and vehicle again. Injections were infused in a volume of 5-μl over a 1-min period, and each injection was separated by 72 hours. Solid bars indicate injection, open bars indicate pre- and post-injection baseline gastric secretion collections. Results are expressed as mmol acid/hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus pre-injection baseline.

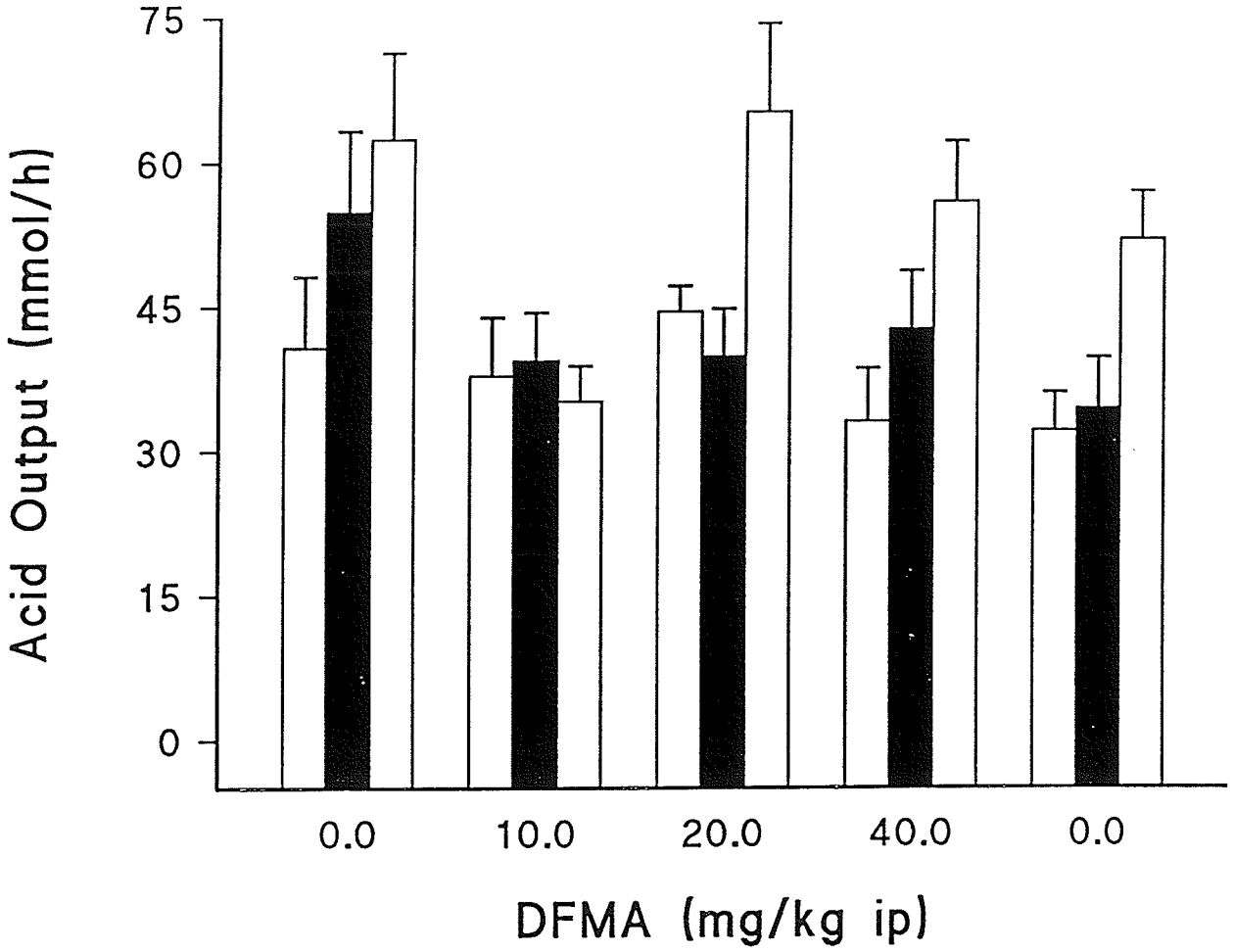

Figure 6: Effect of Peripherally Administered DFMA on Gastric Acid Secretion in the Conscious Rat

Animals (n=6-8) were given individual injections of saline vehicle, DFMA at doses of 10.0, 20.0 and 40.0 mg/kg ip, and vehicle again. Injections (solid bars) were separated by 72 hours. Open bars indicate pre- and post-injection baseline secretion collections. Results are expressed as mmol acid/hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus preinjection baseline.

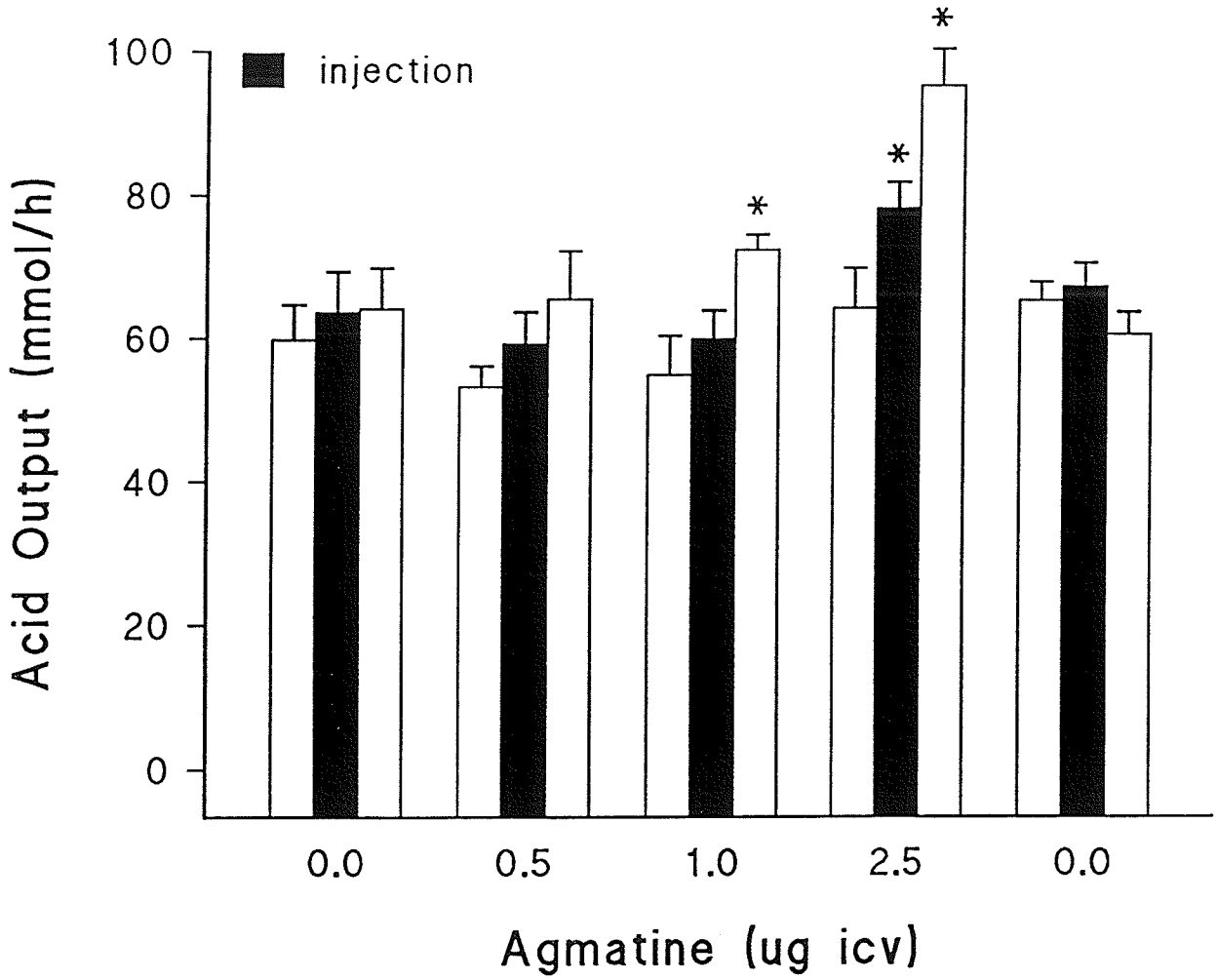

Figure 7: Effect of Centrally Administered Agmatine on Gastric Acid Secretion in the Conscious Rat

Animals (n=6-8) were given saline vehicle, agmatine at doses of 0.5, 1.0 and 2.5 μg icv, and vehicle again. Injections were infused in a volume of 5-μl over a 1-min period, and all injections were separated by 72 hours. Open bars indicate pre- and post-injection baseline gastric secretion collections. Solid bars represent the 1 hour of collection immediately following injection. Results are expressed as mmol acid/hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus pre-injection baseline.

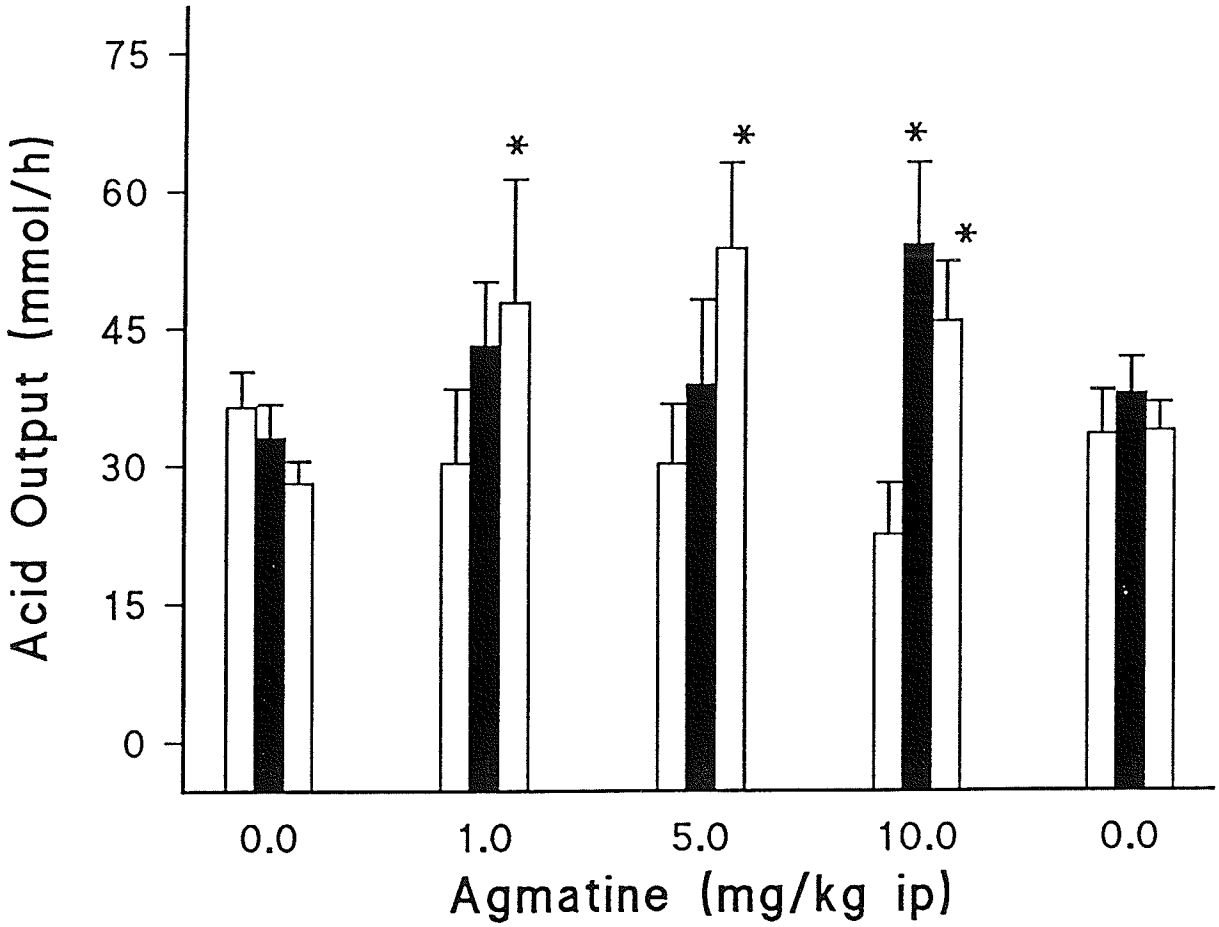

Figure 8: Effect of Peripherally Administered Agmatine on Gastric Acid Secretion in the Conscious Rat

Animals (n=6-8) were given saline vehicle, agmatine at doses of 1.0, 5.0 and 10.0 mg/kg ip, and vehicle again. Injections were separated by 72 hours and are indicated by the solid bars. Open bars indicate pre- and post-injection secretion collections. Results are expressed as mmol acid/hour (mean ± S.E.M.).

* indicates $p < 0.05$ versus pre-injection baseline.

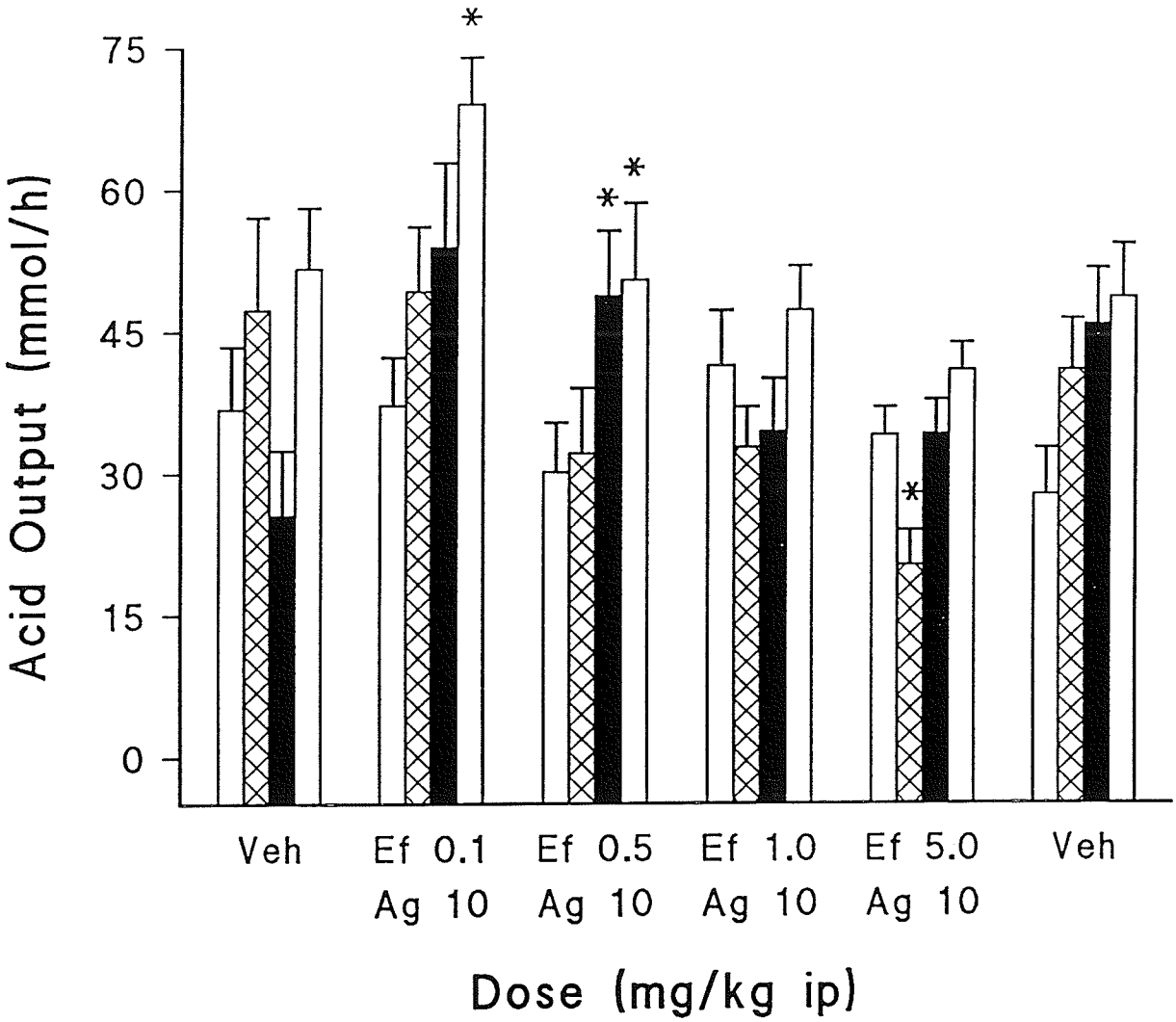

Figure 9: Effect of Efaroxan Pretreatment on the Prosecretory Action of Agmatine

Animals (n=6-8) were pretreated with efaroxan 30 min prior to agmatine administration. Double hatched bars indicate efaroxan administration. Solid bars indicate agmatine administration. Open bars indicate hours in which no injections occurred. Results are expressed as mmol/hour (mean±S.E.M.). * indicates $p<0.05$ vs. hour 1 baseline collection.

Group	Secretion Volume (ml)	Pepsin Output (mg / 4 h)	Pepsin Conc. (mg/ml)	Acid Output (mmol / h)
Vehicle	6.1 ± 0.9	11.1 ± 1.7	2.1 ± 1.7	16.5 ± 1.2
Agmatine 0.1 mg/kg	5.0 ± 0.7	24.6 ± 4.1 *	4.5 ± 0.2 *	17.7 ± 1.1
Agmatine 1.0 mg/kg	7.6 ± 1.1 #	28.4 ± 2.5 *	3.9 ± 0.3 *	21.3 ± 1.0 *#
Agmatine 10.0 mg/kg	7.6 ± 0.3 *#	26.6 ± 1.4 *	3.5 ± 0.1 *	20.2 ± 0.9 *#
Agmatine 20.0 mg/kg	7.8 ± 0.2 *#	27.1 ± 1.6 *	3.6 ± 0.1 *	21.1 ± 1.0 *#

* indicates $p < 0.05$ versus vehicle.
# indicates $p < 0.05$ versus 0.1 mg/kg dose.

Table 5: Effects of Agmatine on Gastric Secretion in Pylorus-ligated Rats

Animals (n=6 per group) were deprived of food for 24 hours. Under anesthesia the pylorus was ligated and the incision closed. Distilled water vehicle or agmatine (0.1, 1.0, 10.0 or 20.0 mg/kg ip) was administered and the animals housed without food or water for 4 hours. They were sacrificed, their stomach contents measured in millilitres, and aliquots taken for determination of acid and pepsin content.

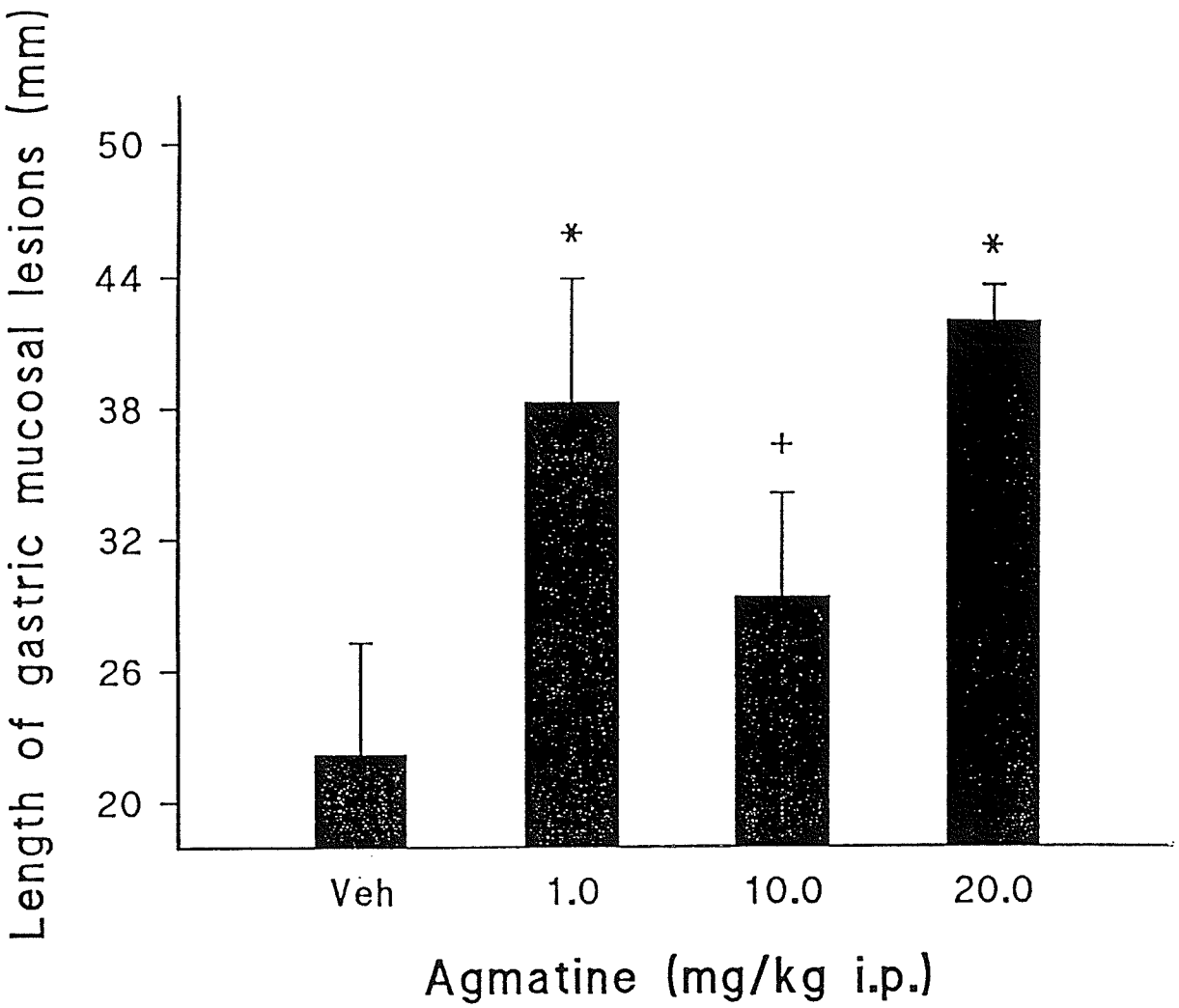

Figure 10: Effect of Agmatine on Gastric Mucosal Injury Induced by Restraint Stress

Animals (n=6 per group) were deprived of food for 24 hours. They were then injected with either saline vehicle or agmatine (1.0, 10.0 or 20.0 mg/kg ip). Following immobilization in a cold (4-6°C) environment for 3 hours, the animals were sacrificed and their stomachs examined for lesions. Ulcer severity was cumulated and expressed in millimeters (mean ± S.E.M.).

* indicates $p<0.05$ versus vehicle.
+ indicates $p<0.07$ versus vehicle.

10.0 mg/kg ip also exacerbated gastric mucosal injury, but this result was not significant ($p < 0.07$). In a second group of animals that was exposed to restraint stress but assayed for gastric adherent mucus concentration, agmatine at 10.0 and 20.0 mg/kg ip significantly reduced mucus levels ($p < 0.05$) (Table 6).

Moxonidine exerted a significant mucopreservative action in animals that underwent restraint stress (Table 7). At all doses (0.01, 0.1, and 1.0 mg/kg ip), moxonidine increased gastric adherent mucus concentration relative to those animals exposed to stress but receiving only saline vehicle ($p < 0.05$). At the highest dose tested (1.0 mg/kg ip), moxonidine augmented gastric mucus production, increasing it beyond those levels observed in animals not exposed to restraint stress. Pretreatment with efaroxan (20.0 mg/kg ip) 30 min before exposure to restraint stress completely obtunded the mucus-preserving action of moxonidine (Table 8). Efaroxan administration alone, at a dose of 20.0 mg/kg ip, had no effect on gastric adherent mucus levels.

Moxonidine at all doses tested (0.1, 0.5, or 1.0 mg/kg ip), significantly reduced the severity of indomethacin-induced antral mucosal lesions ($p < 0.05$) (Figure 11). These results were not dose-related, and as the ED_{50} of moxonidine had previously been calculated to be $\approx$ 0.04 mg/kg ip (Glavin and Smyth, 1995), a ceiling effect for gastroprotection by moxonidine may have been obtained.

Treatment Group	Adherent Mucus (μg/g glandular stomach)
Vehicle	509.3 ± 29.8
Agmatine 0.1 mg/kg	495.8 ± 27.3
Agmatine 1.0 mg/kg	523.7 ± 31.2
Agmatine 10.0 mg/kg	417.8 ± 22.9 *
Agmatine 20.0 mg/kg	440.9 ± 28.5 *

* indicates $p < 0.05$ versus vehicle and 1.0 mg/kg dose.

Table 6: Effect of Agmatine of Gastric Adherent Mucus Levels in Animals Exposed to Restraint Stress

Animals (n=6 per group) were deprived of food for 24 hours. Following saline verhicle or agmatine (0.1, 1.0, 10.0 or 20.0 mg/kg ip) administration, animals were restrained in a cold (4-6°C) environment for 3 hours. They were then sacrificed, their stomachs removed and assayed for gastric adherent mucus concentration. Results are expressed as μg/g glandular stomach tissue (mean ± S.E.M.).

Treatment Group	Adherent Mucus (μg/g glandular tissue)
Vehicle - no stress	422.5 ± 16.1
Vehicle - stress	288.0 ± 12.3
Moxonidine 0.01 mg/kg	442.9 ± 24.1 *
Moxonidine 0.1 mg/kg	314.4 ± 20.1 *
Moxonidine 1.0 mg/kg	353.9 ± 17.5 *

* indicates $p < 0.05$ versus vehicle-stress group.

Table 7 : Effect of Moxonidine on Gastric Adherent Mucus Levels in Animals Exposed to Restraint Stress

Animals (n=8 per group) were deprived of food for 24 hours. Following administration of either saline vehicle or moxonidine (0.01, 0.1 or 1.0 mg/kg ip), they were restrained in a cold (4-6°C) environment for 3 hours. Following sacrifice, their glandular stomachs were weighed and assayed for gastric adherent mucus. Results are expressed as μg/g glandular stomach tissue (mean ± S.E.M.).

Treatment Group	Adherent Mucus (μg/g glandular tissue)
Vehicle - vehicle - no stress	458.9 ± 26.2
Vehicle - vehicle - stress	209.1 ± 22.7
Efaroxan 20.0 mg/kg - Vehicle	225.8 ± 21.9
Efaroxan 20.0 mg/kg - Moxonidine 0.01 mg/kg	231.1 ± 23.5
Efaroxan 20.0 mg/kg - Moxonidine 0.1 mg/kg	244.6 ± 26.3
Efaroxan 20.0 mg/kg - Moxonidine 1.0 mg/kg	239.4 ± 29.1

* indicates $p < 0.05$ versus vehicle - vehicle - stress group.

Table 8: Effect of Efaroxan Pretreatment on Moxonidine-induced Preservation of Gastric Adherent Mucus in Animals Exposed to Restraint Stress

Animals (n=6 per group) were deprived of food for 24 hours. They were pretreated with either saline vehicle or efaroxan (20.0 mg/kg ip), followed 30 minutes later by a second saline vehicle or moxonidine (0.01, 0.1 or 1.0 mg/kg ip). After 3 hours of immobilization in a cold (4-6°C) environment the animals were sacrificed. Their glandular stomachs were removed, weighed and assayed for gastric adherent mucus concentration. Results are expressed as μg/g glandular stomach tissue (mean ± S.E.M.).

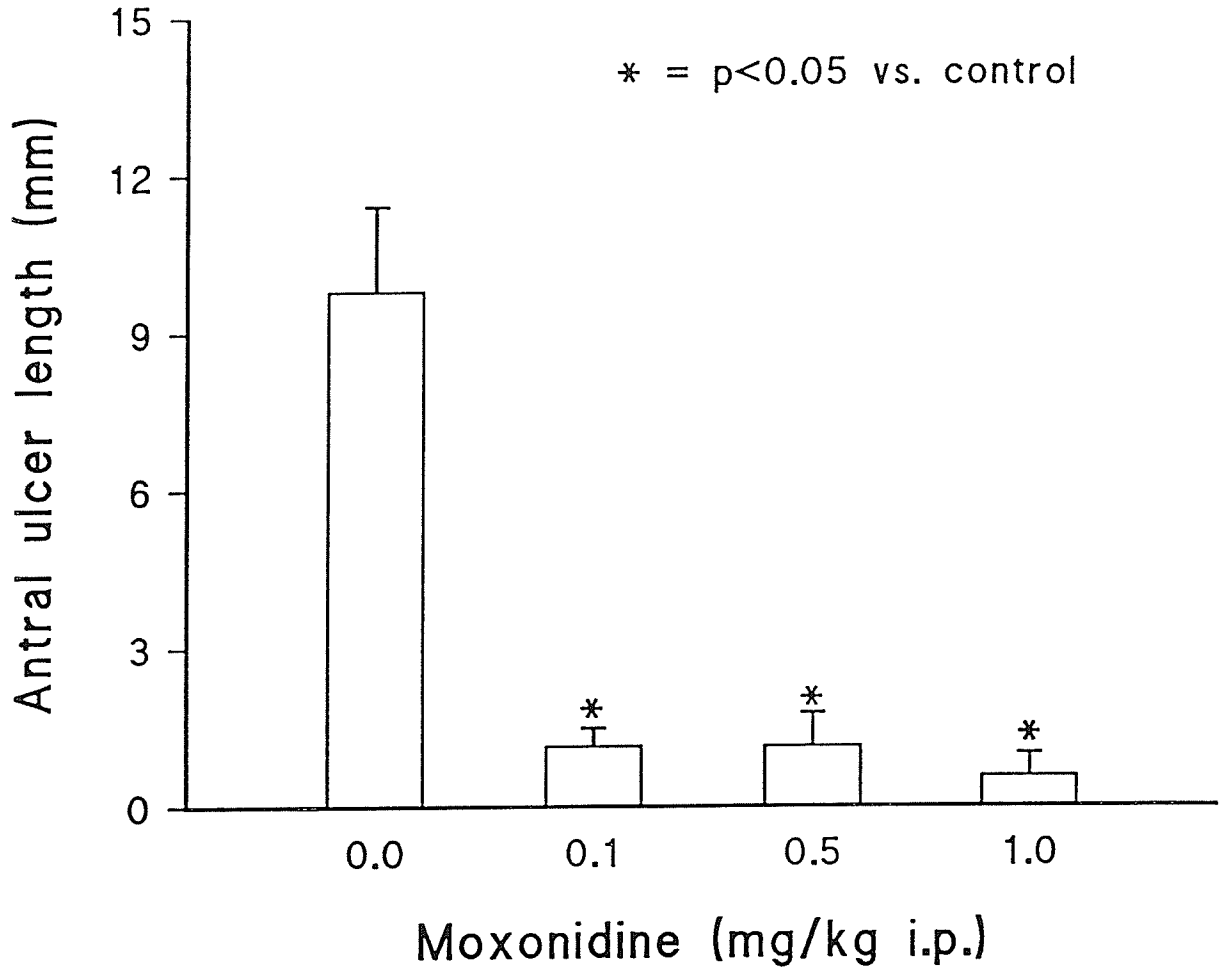

Figure 11: Effect of Moxonidine on Indomethacin-induced Antral Lesions

Animals (n=10 per group) were deprived of food for 24 hours. They were allowed 1 hour free access to food after which time they were injected with indomethacin (32.0 mg/kg sc). Immediately following indomethacin administration, they were given either saline vehicle or moxonidine (0.01, 0.1 or 1.0 mg/kg ip). Six hours later the animals were sacrificed and their stomachs removed. Antral ulcer severity was cumulated and results are expressed in millimeters (mean ± S.E.M.).

* indicates $p < 0.05$ versus vehicle.

DISCUSSION

Recent reports have demonstrated that the antihypertensive actions of clonidine are due to its interaction with I_1-imidazoline receptors within the rostral ventrolateral medulla (Ernsberger et al., 1987). Results showing an effect of clonidine on gastrointestinal function however, have been inconsistent. Following instillation of ethanol, clonidine produced a biphasic response on gastric mucosal injury (Bhandare et al., 1991). At low doses clonidine was protective, at high doses it exacerbated ethanol-induced lesions. Yohimbine pretreatment was able to block the gastroprotective action of the 0.1 mg/kg dose of clonidine, suggesting that clonidine was acting at predominately α_2-receptors. At a dose of 1.0 mg/kg, clonidine's exacerbatation of mucosal injury was not blocked by yohimbine pretreatment, suggesting the possibility of imidazoline receptor involvement. The extent to which clonidine's actions on gastrointestinal function are mediated by the imidazoline receptor is not yet known. However, with the development of selective I_1-agonists such as moxonidine, it has become possible to explore the role imidazoline receptors play in the physiology of the gut.

Glavin and Smyth (1995) have shown peripherally administered moxonidine, a selective I_1-imidazoline agonist, to be gastroprotective against ethanol-induced mucosal damage and antisecretory in both anesthetized and conscious animals. The present data show that in measures of conscious gastric acid secretion, moxonidine administered centrally exerted a dose-dependent antisecretory effect. At the highest dose administered (5μg icv), acid output was reduced by 38% when compared to pre-injection baseline controls. These results parallel those of Glavin and Smyth (1995); however, the magnitude of inhibition of

gastric acid secretion was greater following peripheral administration of moxonidine. When comparing relative changes in acid output during the second hour following drug administration, centrally administered moxonidine maximally inhibited gastric acid secretion by 38% whereas peripherally administered moxonidine's maximum inhibition reached 100%. These data suggest a greater role for peripheral as compared with central I_1-imidazoline receptors in mediating gastrointestinal function.

Further evidence supporting this observation follows from experiments measuring gastric acid output in the conscious rat in response to efaroxan pretreatment in combination with moxonidine administration. Centrally administered efaroxan was not able to block the antisecretory actions of peripherally administered moxonidine; nor was peripherally administered efaroxan able to block the antisecretory effect of centrally administered moxonidine. The only injection combination able to block moxonidine's antisecretory effect was one in which efaroxan pretreatment was given peripherally, followed by moxonidine given peripherally. The inhibition in acid output seen at a 1.0 mg/kg ip dose of moxonidine, a dose previously shown to be maximally antisecretory (Glavin and Smyth, 1995), was blocked by efaroxan in a dose-dependent fashion. When both compounds were administered centrally, a similar inhibition of acid output was not observed (Carlisle et al., unpublished observations). These results add further support to a role for I_1-imidazoline receptors in gastrointestinal function, as the effects of a selective I_1-imidazoline agonist (moxonidine) could be antagonized by a selective I_1-imidazoline blocker (efaroxan).

Recent trends within the field of peptic ulcer disease research have focussed on developing agents which augment the defensive factors involved in gastrointestinal mucosal

protection. The present results, as well as those of Glavin and Smyth (1995), show moxonidine to be protective in a number of experimental models of gastric mucosal injury. It reduces the severity of ethanol- and indomethacin-induced lesions, and augments adherent mucus secretion in animals exposed to cold-restraint stress. Moxonidine also raised intragastric pH suggesting the augmentation of bicarbonate secretion, although this was not confirmed by experimentation (Glavin and Smyth, 1995). Evaluation of the gastrointestinal effects of moxonidine therefore, suggests that I_1-imidazoline receptor activation may provide a novel mechanism of gastroprotection (Carlisle et al., 1995).

Gastroprotection in the face of mucosal insult is a multifactoral process; thus there are many possible mechanisms through which moxonidine may be exerting its protective actions. Given that a proposed signal transduction pathway for I_1-imidazoline receptors is via the activation of phospholipase A_2 (Ernsberger et al., 1995), it can be hypothesized that some of moxonidine's observed protective effects may be due to the generation of prostaglandins. Ernsberger et al. (1995) found that *in vitro,* moxonidine administration increased prostaglandin E_2 formation five-fold. In the kidney, I_1 - receptor activation increases urine flow rate secondary to an increase in osmolar clearance (Allan et al., 1993). Darkwa and Smyth (1995) observed that after indomethacin pretreatment, the diuretic and natriuretic effects of moxonidine were abolished. When prostaglandin E_2 was co-administered with indomethacin however, moxonidine's renal effects were restored (Darkwa and Smyth, 1995).

If the signal transduction pathway associated with the I_1-receptor is due to phospholipase A_2 activity, and physiologically relevant concentrations of prostaglandins were

to be generated within the gastric mucosa, this might account for some of moxonidine's ability to preserve gastric adherent mucus during cold-restraint stress. This would not imply however, that a prostaglandin-induced increase in gastric adherent mucus levels causes the gastroprotection seen following moxonidine administration. It has been shown that there is no correlation between gastric mucosal protection by prostaglandins and the thickness of the mucus barrier (Szabo, 1991).

In doses which do not alter acid secretion, exogenous prostaglandin administration has been shown to be protective against ethanol-induced lesions (Robert et al., 1979). Although the surface epithelium is damaged following such an insult, the underlying mucosal layers remain undamaged. Although a variety of vasoactive agents are involved in controlling gastric mucosal blood flow, endogenous prostaglandin generation and the resultant maintenance of gastric mucosal blood flow may prove to be a more important mechanism through which moxonidine is acting. The sequelae of maintained gastric mucosal blood flow are many. Adequate blood flow is required to provide the delivery of those substrates required for the process of rapid epithelial restitution in response to superficial mucosal damage. Blood flow is also involved in the systemic and local delivery of bicarbonate, as well as in the removal of back-diffused acid.

Although there is a correlation between portal hypertension and peptic ulcer disease, it is not known if portal hypertension plays a direct role in ulcer diathesis. Propranolol administration in doses which lower portal pressure, protects against ethanol-induced lesions in the portal hypertensive animal (Sankary et al., 1986), and has been shown to reduce both variceal bleeding and gastrointestinal hemorrhage in portal hypertensive patients (Lebrec et

al., 1981). Propranolol improves mucosal hemodynamics by a mechanism which may be due in part, to prostaglandin production (Bhandare et al., 1990). Studies involving moxonidine administration in experimental portal hypertension have not been undertaken; however, the hemodynamic and gastrointestinal actions of this agent may have implications for the treatment of patients suffer from both peptic ulcer disease and portal hypertension.

Indomethacin administration is a common method for inducing mucosal lesions experimentally. The inhibition of cyclooxygenase and the resultant decrease in prostaglandin production is a purported mechanism by which mucosal injury occurs. Our data show that moxonidine was protective against indomethacin-induced lesions, and an increase in prostaglandin production with concomitant maintenance of gastric mucosal blood flow cannot be ruled out as the protective mechanism. However, the location and degree of lesion formation has been shown to depend on the feeding conditions prior to indomethacin administration. Satoh et al. (1981), reported that while indomethacin administration reliably produces corpus lesions in the fasted rat, in the refed rat ulcers are seen primarily in the antrum, with few corpus lesions. Although exogenous prostaglandin administration prevents lesion formation (Lippman, 1974; Satoh et al., 1981), it is clear that other factors related to feeding are superimposed on the ulcerative process. Kuratani et al. (1992), proposed that this factor might be post-prandial hyperinsulinemia which induces increased sympathetic outflow and vasoconstriction. Its vascular hemodynamic effects aside, if this hypothesis is true, then moxonidine may also be acting to alter insulin release from pancreatic islet cells, since it is known to inhibit insulin release from isolated pancreatic islet cells (Tsoli et al., 1995).

Given these observations with moxonidine, it would be expected that administration

of the putative endogenous imidazoline receptor ligand (Li et al., 1994), would produce similar effects on gastrointestinal function in each of our experimental protocols. Following central administration, agmatine augmented gastric acid output, to a maximum of 44%. Upon peripheral administration, agmatine, at all doses tested, significantly increased acid secretion. Maximum potentiation of acid output equalled 140% relative to pre-injection baseline controls. The prosecretory effects of agmatine occurred to a greater extent in the second hour following drug administration. This prosecretory effect was also seen in anesthetized animals, as both acid and pepsin outputs increased in pylorus-ligated animals who received agmatine at doses of 1.0 mg/kg to 20.0 mg/kg ip. These results do not reflect a dose-related phenomenon however, possibly because the ED_{50} of agmatine is a value closer to the lower doses in the range we tested. Therefore, the absence of a dose-dependent effect might actually be due to binding site saturation at the higher doses in our test range. Similar trends towards an opposing effect of agmatine as compared to moxonidine followed agmatine administration in animals exposed to cold-restraint stress. Agmatine exacerbated the severity of lesions and had a significant effect on reducing gastric adherent mucus levels.

Data from the above experiment, leave doubt as to the exact nature of agmatine's activity as an imidazoline receptor ligand. It would appear that the activation of the I_1-imidazoline receptor is associated with potent gastroprotective and antisecretory effects, as evidenced by experiments involving administration of moxonidine, a highly selective I_1-imidazoline receptor agonist. As agmatine produced physiological effects which were diametrically opposed to those of moxonidine, it is possible that agmatine may not be as selective for the I_1-imidazoline receptor as has been previously reported. However, in

measurements of basal acid output, efaroxan antagonized the effects of both moxonidine and agmatine, suggesting that these three compounds are acting at the same site. Data concerning the antagonism of agmatine's actions by efaroxan are preliminary, and further experiments need to be undertaken. Gumusel et al. (1995) for example, have reported that the vasodilatory response following systemic agmatine administration was not antagonized by efaroxan. As agmatine also binds to I_2- receptors, some of its gastrointestinal actions may be due to its interaction at this site. This could account for the dissimilarity of action between moxonidine and agmatine.

Given these differential results on gastrointestinal function, it has been suggested that agmatine is acting as an inverse agonist at imidazoline receptors (Glavin et al., 1995). Observations of opposing actions for these two agents has not been limited to the gastrointestinal tract. Agmatine has hypoglycemic properties and has been used in clinical practice as an anti-diabetic agent (Morgan et al., 1995), whereas moxonidine inhibits insulin release (Tsoli et al., 1995). Intracisternal moxonidine administration is hypotensive (Head, 1995), while agmatine has been shown to exert a dose-dependent increase in arterial pressure after intracisternal administration (Sun et al., 1995). However, the effects of agmatine on blood pressure vary depending on the route of administration. Microinjection of agmatine into the rostral ventrolateral medulla, the site of moxonidine's antihypertensive effects, has been shown to have no effect on blood pressure or sympathetic outflow, while systemic administration decreased arterial pressure probably by its acting as a vasodilator (Sun et al., 1995).

Agmatine concentrations have been assayed in various tissues and found to be

especially high within the stomach (Raasch et al., 1995). This would suggest that agmatine has an important regulatory function within the gastrointestinal tract. However, neither peripherally- nor centrally-administered efaroxan, at doses which blocked both moxonidine's antisecretory and agmatine's prosecretory effects, produced significant changes in basal acid output. Similarly, efaroxan administered as the sole experimental agent, produced no changes in gastric adherent mucus levels in animals exposed to cold-restraint stress. These results suggest that agmatine, if it is an agonist at the I_1-receptor, is not tonically active. Similarly, administration of the arginine decarboxylase inhibitor DFMA, in each of our our secretion models, did not significantly alter acid output. Whereas, dose-response relationships are well-established for difluoromethlyornithine (DFMO), the ornithine decarboxylase inhibitor (Wang and Johnson, 1994), relatively little is known about the doses required to effectively inhibit arginine decarboxylase. The doses of DFMA used in the above studies were, in comparison, much lower than those normally used to inhibit ornithine decarboxylase. Therefore, we cannot eliminate the possibility that agmatine has tonic activity in the gastrointestinal tract until comprehensive dose-response relationships to arginine decarboxylase inhibition are established.

Until the discovery that agmatine was a clonidine-displacing substance, it was believed that agmatine served only as a precursor to polyamine synthesis. It has since been discovered that agmatine's bioactive properties are many and varied. In each of our laboratory's experimental models, agmatine exerted a gastrointestinal action after acute administration. These effects were most likely due to an imidazoline receptor-dependent mechanism. However, agmatine has always been considered a precursor to the polyamines by a synthetic

route secondary to the l-ornithine pathway (Figure 12). Polyamines function within the gastrointestinal tract to mediate mucosal lesion healing following an acute insult, such as exposure to water-immersion stress, (Wang and Johnson, 1994). It is possible that agmatine administration, especially on a chronic basis, could serve to provide excess precursor for polyamine synthesis, thus acting in a manner independent from the imidazoline receptor. Similarly, the relationship between nitric oxide and agmatine should be explored further, given that they share a common precursor. Nitric oxide, in a mechanism that most likely involves the maintenance of gastric mucosal blood flow, is protective against a number of mucosal-damaging factors, including topical ethanol (Masuda et a., 1995).

The debate as to whether or not agmatine is the putative endogenous ligand of the imidazoline receptor aside, the fact that its precursor molecule forms two other substances with important biological effects in the gut is reason enough to explore the properties of agmatine. Selective inhibitors of each of the synthetic enzymes in the l-arginine-agmatine-nitric oxide-ornithine pathway are now available. Therefore, not only is it important, but it is now possible, to study the relative contributions of each of these molecules in mediating gastrointestinal function and responses to injury. More specifically, how does exogenous administration of excessive substrate or direct enzyme inhibition, influence the synthesis of the others factors in the pathway?

In summary, peripheral I_1-imidazoline receptor activation is associated with significant antisecretory and antiulcer effects, and may provide a novel mechanism for gastroprotection. As a proposed signal transduction pathway following I_1-agonist binding is phosopholipase A_2 activation and the generation of prostaglandins, and since prostaglandins remain clinically

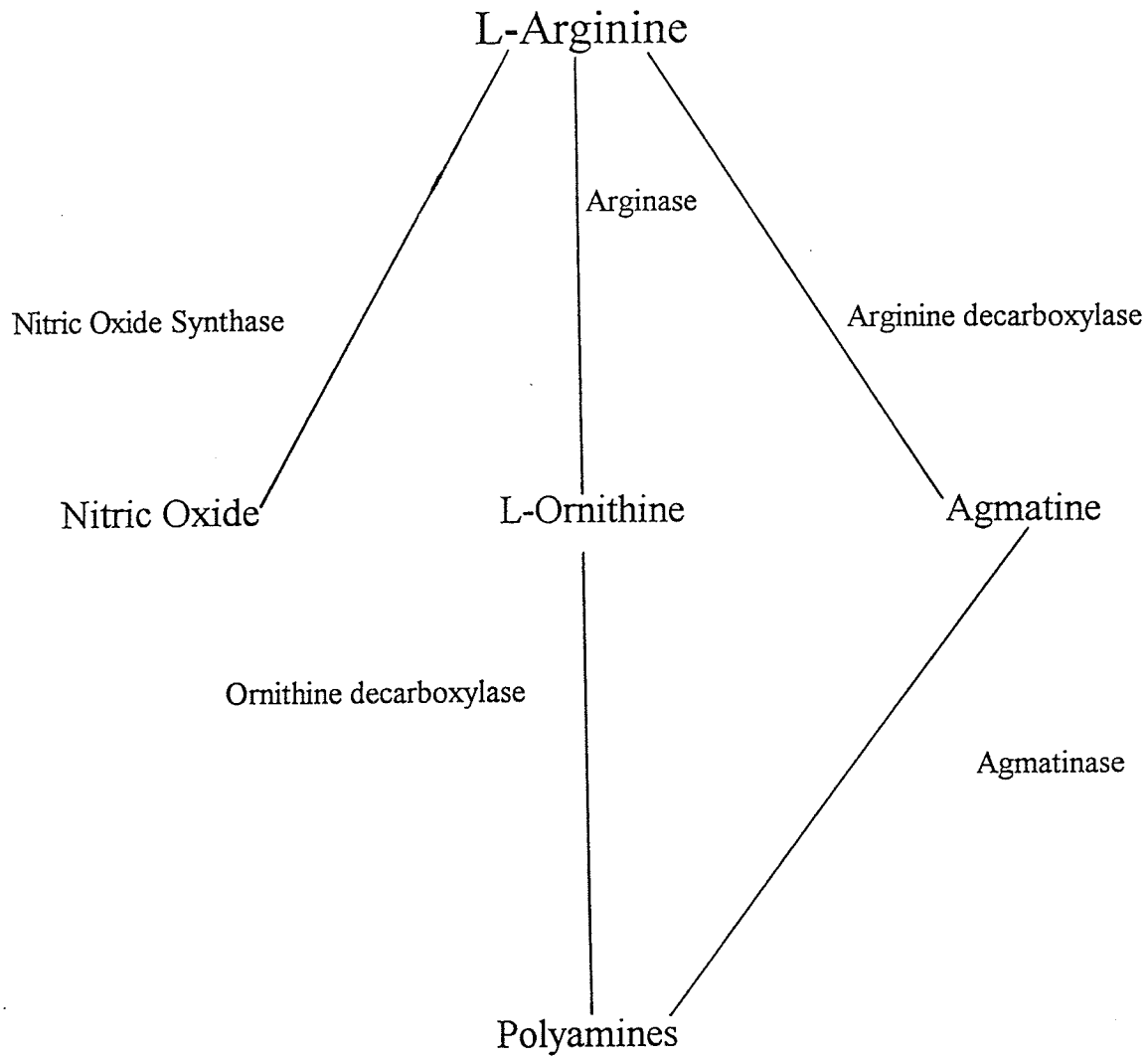

Figure 12: Agmatine Synthesis

useful in ulcer therapy, the implications for both basic research and possibly clinical treatment of peptic ulcer are exciting. In contrast, agmatine administration produced effects on gastrointestinal function which were opposite to those seen with moxonidine. These contrasting data lead to the speculation that agmatine may be acting at a receptor other than the I_1 site, or that it may be acting as an inverse agonist at this site. Finally, given their common precursor, the interaction between agmatine and both polyamines and nitric oxide should be explored further, especially since all of the products within that synthetic pathway have now been shown to be bioactive and can mediate aspects of gastrointestinal function.

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