PERIPHERAL MECHANISMS INVOLVED IN THE GASTRIC RESPONSES TO CENTRAL VAGAL STIMULATION
(Ph.D. THESIS)

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The PhD Thesis is Dedicated to my Mother, Father, Husband and Daughter

LIST OF ABBREVIATIONS

CGRP	Calcitonin gene-related peptide
CNS	Central nervous system
CSF	Cerebrospinal fluid
DMN	Dorsal motor nucleus of the vagus
DVC	Dorsal vagal complex
GI	Gastrointestinal
GMBF	Gastric mucosal blood flow
GMVR	Gastric mucosal vascular resistance
IC	Intracisternal
IG	Intragastric
IP	Intraperitoneal
IV	Intravenous
L-NAME	N-nitro-L-arginine methyl ester
L-NMMA	N_G-monomethyl-L-arginine
MAP	Mean arterial pressure
MMC	Mucosal mast cell
MP	Myenteric plexus
NANC	Non-adrenergic non-cholinergic
NO	Nitric oxide
NS	Not significant
NTS	Nucleus of the solitary tract
RMCPII	Rat mast cell protease-II
SC	Subcutaneous
SEM	Standard error of mean
TRH	Thyrotropin releasing hormone
TRH-LI	Thyrotropin releasing hormone-like immunoreactivity
VIP	Vasoactive intestinal polypeptide

CONTENTS

1. GENERAL INTRODUCTION

Considerable attention over the years has been directed toward understanding the relationship between the central nervous system (CNS) and gastrointestinal (GI) ulceration. In particular the common association of stress-related gastric mucosal damage with CNS injury has supported the hypothesis that the CNS may play a significant role in controlling the structural integrity of the GI mucosa. It is well known that after intracranial diseases, head trauma, or CNS surgery patients are often afflicted with GI hemorrhage and acute ulceration known as "Cushing ulcer". Cushing in 1932 suggested such ulceration to be a result of vagal hyperactivity (Cushing, 1932) and gastric acid hypersecretion. This hypothesis has been further supported by the observation of increased gastric acid secretion in patients with CNS injury, an effect that is thought to be mediated by through effect on vagal nuclei in the medulla (Beattie, 1932; Cushing, 1932) and centers in the hypothalamus (Heslop, 1938). The establishment of the presence of a family of peptide in both the gut and the brain led to the speculation that these peptide may act as CNS neurotransmitters involved with the control of GI function. Studies examining the effects of intracerebral administration of such brain-gut peptide have demonstrated specific effects on gastric functions.

The vagus nerve with its sensory and preganglionic parasympathetic innervation to the abdominal viscera has long been in the focus of treatment for gastric ulceration considering its influence on gastric acid secretion. The presence of gastric acid was supposed to be required for the development of gastric erosions, thus the elimination of both efferent and afferent vagal effects on acid secretion through vagotomy has been widely used in gastric and duodenal ulcer therapy.

Based on the demonstration by Mózsik *et al.* that truncal vagotomy in turn aggravates gastric mucosal damage although the

gastric acid secretion has been suppressed, the role of parasympathetic nervous system in the gastric lesion formation was widely investigated in acute and chronic vagotomized rats (Singh, 1980; Mozsik 1991; Cho 1992; Kiraly 1992; Mozsik 1992a; Karadi 1994). Several sets of experiments suggested a key role of vagal nerve in controlling GI integrity. We have shown that surgical vagotomy not only aggravates the gastric lesion formation in different experimental conditions (ethanol model-(Mozsik 1991) , indomethacin model-(Karadi 1994)) but also abolishes the gastric cytoprotection induced by prostaglandins, retinoids, acid inhibitors (omeprazole-(Cho 1992), cimetidinie-(Mozsik 1991; Cho 1992), atropine-(Mozsik 1991)) and adaptive cytoprotection (Henegan 1984). The unique ability of prostaglandins, in particular PGE_2, to prevent gastric mucosal injury elicited by a variety of experimental conditions independently of changes in gastric acid secretion was established by Robert *et al* (Robert 1978) and Chaudhury *et al* (Chaudbery 1978). The attenuation of antiulcer action of misoprostol and omeprazole was reported in vagotomized rats by using *ex vivo* stomach chamber technique (Cho 1992). These studies point to the importance of the vagal nerve in gastric cytoprotection by compounds eliciting their cytoprotective activity in different way (b-carotene - scavenger (Javor 1983; Mozsik 1986), PGI_2 - gastric mucus, bicarbonate secretion, promotion of repair mechanisms (Robert 1978) etc).

Therefore the effects of acute and chronic surgical vagotomy on gastric functions and defensive mechanisms (blood flow (Cho 1992) gastric emptying (Mozsik 1992a), vascular permeability (Kiraly 1992), mucosal prostaglandin production (Bodis 1990; Suto 1992)) of the stomach were widely investigated by us.

1.1 TRH in the dorsal vagal complex (DVC) stimulates vagal outflow to the stomach and gastric functions

Although multitude of chemical systems in the CNS can influence different gastric functions through vagal dependent mechanisms, still little is known about the physiological role of these transmitters in the vagal regulation of gastric function. During the last decade, much attention has been focused on the three amino acid peptide, thyrotropin-releasing hormone (TRH). TRH was the first brain peptide shown to affect gut function after central administration (Tache 1980). When TRH was first identified in 1970, it was assumed the it served a specific function in the hypothalamo-hypophyseal axis.

The initial report that TRH potently stimulates central vagal dependent gastric acid secretion in rats (Tache 1980) was followed by growing neuroanatomical and neuropharmacological information on central TRH actions influencing GI functions through vagal pathways in different species of experimental animals (Goto 1985; Tache 1988; Ishikawa 1988; Tache 1994a). Furthermore it was clearly shown that TRH action in the medulla completely independent of its hypophysiotropic action (Tache 1980).

1.1.1 Distribution of TRH-like Immunoreactivity (TRH-LI) and TRH Receptors in the Dorsal Vagal Complex (DVC)

The distribution of THR-like immunoreactivity (TRH-LI) in the brain stem supports an important role of the tripeptide in the regulation of vagal activity to the stomach. Nearly 65% of total medullary TRH-LI is located in nuclei associated with the dorsal vagal complex (DVC, composed of the dorsal motor nucleus of the vagus (DMN) and the nucleus of the solitary tract (NTS)) (Eskay 1983; Hornby 1989). In the DVC, TRH-LI is found exclusively in varicose and nonvaricose segments of unmyelinated

axons and in presynaptic terminals (Brownstein 1974; Tache 1994a).

Electronmicroscopy studies have shown that gastric preganglionic neurons contain rich dendritic arborization which reach the nucleus subgelatinosus in the medial NTS and make monosynaptic contact on gastric vagal sensory neuron terminals providing the basis of vago-vagal reflex (Rinaman 1989; Tache 1994a). Double-labeling techniques performed in rats have demonstrated the existence of direct asymmetric synaptic contacts (indicative for excitatory synapse) between TRH-LI containing terminals and dendrites of gastric motoneurons coursing throughout the DMN and medial NTS mainly in the subnucleus gelatinosus immediately caudal to the obex (Eskay 1983). In addition, the presence of nonsynaptic TRH-LI varicosities suggests a possible role of paracrine neuromodulating role in the DVC. Such localization of TRH-LI in the DVC is not unique to rats and has also been reported in the cat medulla (Hornby 1989).

The distribution of TRH receptors in the DVC well correlates with the presence of TRH-LI in the nerve terminals. The highest concentration of TRH receptors is located in the medial part of the DMN containing preganglionic vagal neurons innervating the stomach and influencing gastric function (Laughton 1987; Wu 1992).

TRH receptors are also found in the lateral column of the DMN contributing to the celiac branch of the vagus, as well as in the nucleus subgelatinosus, medial subnuclei of the NTS, and area postrema (Wu 1992). The presence of TRH receptors in neurons of the DMN and NTS was further established by in situ hibridisation technique using mRNA coding for mice pituitary TRH receptor (Wu 1992).

1.1.2 Excitatory Effect of TRH on DMN Motorneurons and Vagal Efferent Activity

The morphological demonstration of asymmetric synapses indicates functional excitatory input between TRH-LI presynaptic terminals and gastric motoneuronal dendrites which was confirmed by electrophysiological studies (McCann 1989; Rogers 1989; Ragenbass 1990). TRH increases the firing rate of DMN neurons either in vivo or in vitro (McCann 1989; Rogers 1989; Ragenbass 1990). The response assessed in vitro was not altered by inhibition of synaptic transmission showing that TRH exerts a direct postsynaptic excitatory effect on DMN neurons (Ragenbass 1990). Regarding the influence of TRH on NTS neurons, an inhibitory effect was reported in vivo on neurons responding to gastric inflation (Rogers 1989). By contrast in an in vitro slice tissue preparation, TRH consistently induced a reversible excitatory effect which persists in the presence of blockade of synaptic transmission (Ragenbass 1990). Whether the inhibitory action of TRH on NTS neurons in vivo represents an indirect action needs to be further investigated.

TRH applied directly to the DMN was shown to increase the parasympathetic outflow to various viscera. Injection of TRH or stable TRH analog, RX 77368 [p-Glu-His-(3,3'dimethyl)-Pro-NH_2], in the cerebrospinal fluid (CSF) stimulates vagal efferent activity recorded from the cervical, gastric, superior laryngeal, and ciliar branches of the vagus (Somiya 1984; O-Lee 1997).

1.1.3 TRH in the DMN Induces a Vagal Dependent Stimulation of Gastric Function

Microinjection of TRH or the stable TRH analog RX 77368, in the DMN induced a vagal dependent stimulation of gastric acid and pepsin secretion and gastric mucosal blood flow (GMBF) in

anesthetized rats (Tache 1980; Tache 1988; Ishikawa 1988; Thiefin 1989; Yanagisawa 1990a; Yang 1992; Kiraly 1994) or cats (Feng 1990). Based on the biological response the DMN seems to be more responsive than any other medullary site (Tache 1994a). Microinjection of TRH or RX 77368 in the DMN also induced a vagal muscarinic stimulation of gastric motor function assessed by an increase in corpus tone and phasic high-amplitude contractions, and antral and pylorus contractility in rats (Madea-Hagiwara 1987; Garrick 1987; Tache 1994a) and cats (Rinaman 1989).

1.2 Influence of central vagal activation by TRH on gastric secretion: functional implications

1.2.1 Increase in Gastric Secretion

1.2.1.1 Histamine

There is an evidence that intracisternal injection of TRH stimulates histamine release as assessed by the increase of histamine levels in the portal blood or gastric interstitial fluid or fundic submucosa in anesthetized rats (Fig. 1) (Yanagisawa 1990a; Tache 1994a). The portal release of histamine induced by central TRH analog is mediated by vagal muscarinic pathways independently from gastrin (Yanagisawa 1990a).

1.2.1.2 Serotonin

Serotonin concentration of the portal blood and fundic submucosa is markedly increased for sustained periods of time upon injection of TRH or stable analog in the cisterna magna or DMN (Stephens 1989; Yang 1992). The stimulation of gastric serotonin by central injection of RX 77368 is mediated by vagal muscarinic pathways and is not related to the increase in acid secretion (Stephens 1989; Yang 1992) (Fig. 1).

The relative contribution of the various cellular pools of gastric serotonin in the luminal release is not clear. Gastric stores of serotonin are localized in endocrine (enterochromaffin

and "enterochromaffin-like" cells), mast cells and enteric neurones (Stephens 1989). Rat gastric enterochomaffin-like cell readily synthesize and store serotonin, in addition , gastric mast cell degranulation has been correlated with the increased intraluminal serotonin release (Stephens 1989; Yang 1992; Tache 1994a) and decreased mast cell serotonin levels.

1.2.1.3 Prostaglandins

Early studies demonstrated that direct electrical stimulation of the vagus nerve increases PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ levels in venous and luminal effluent of totally isolated vascularly perfused rat stomach (Singh, 1980). Similarly acute surgical vagotomy decreased the gastric mucosal content of 6-keto-$PGF_{1\alpha}$, and PGI_2 (Bodis 1990; Suto 1992).

Intracisternal injections of TRH or RX 77368 induced a dose-related release of PGE_2 (Fig. 1) which is mediated through vagal muscarinic mechanisms not related to the stimulation of acid secretion (Yoneda 1993b). Bilateral cervical vagotomy completely prevented PGE_2 release into the interstitial fluid of the corpus submucosa (Yoneda 1993b) after central RX 77368. The endogenous gastric prostaglandins are biologically active to suppress gastric acid secretion when submaximal doses of TRH are used (Yoneda 1993b).

1.2.1.4 Nitric Oxide

Central vagal activation by TRH analog stimulates nitric oxide (NO) (Kiraly 1993; Tanaka 1993) release (Fig. 1) from the endothelium in blood vessels of the gastric mucosa. This is supported by the demonstration that the stimulation of gastric mucosal blood flow or gastric cytoprotection induced by intracisternal injections of TRH analog are completely prevented by the nitric oxide synthesis inhibitor, N-nitro-L-arginine methyl ester (L-NAME) (Tanaka 1993).

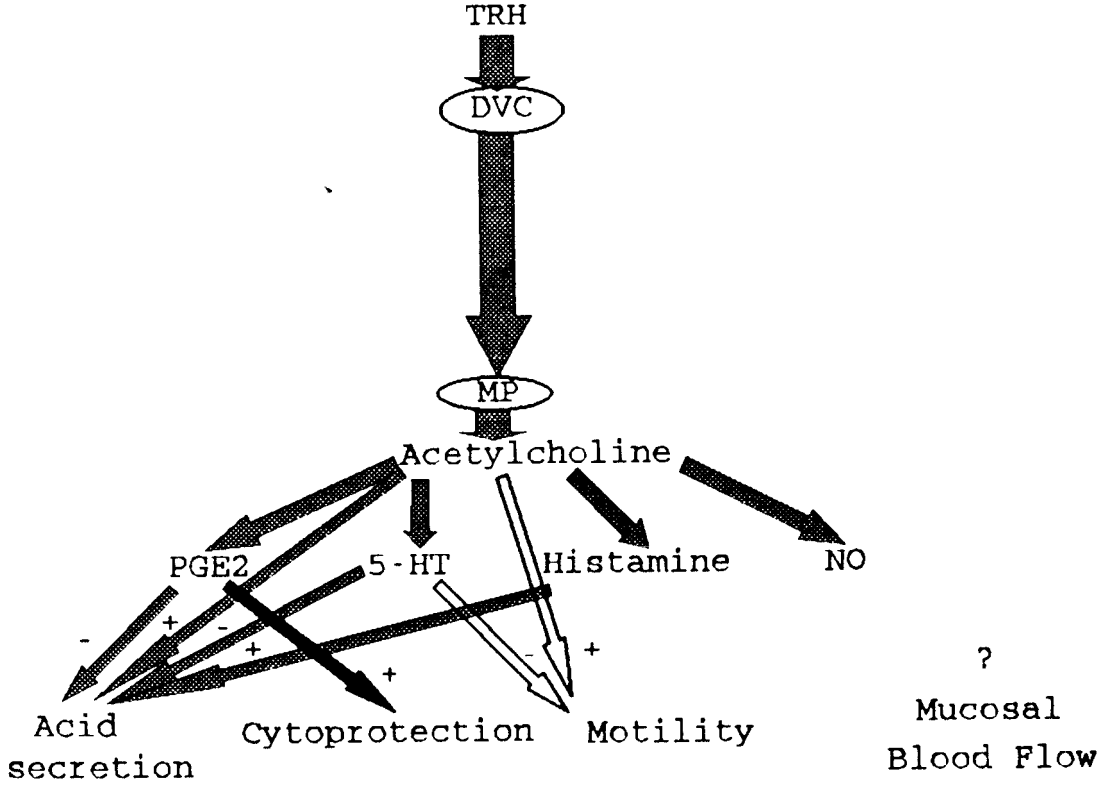

Fig. 1. Schematic illustration of the activation of dorsal vagal complex (DVC) and peripheral action of thyrotropin releasing hormone (TRH) injected intracisternally (myenteric plexus (MP), prostaglandin F_2 (PGF_2), 5-hydroxy-tryptamine (5-HT), nitric oxide (NO))

1.2.2 Functional Implications

1.2.2.1 Acid Secretion

A stimulation of gastric acid secretion was suggested after central injection of TRH or stable TRH analog in a dose-dependent manner (Tache 1980; Ishikawa 1988; Yang 1990). The site of action of TRH or stable analog was suggested to be in the nucleus ambiguus and DVC or raphe pallidus (Ishikawa 1988; Yang 1990). These results also suggest a role for these nuclei and TRH in the medullary regulation of gastric acid secretion.
At maximal effective dose (30 ng), more than half of the acid response is mediated through vagal muscarinic release of gastric histamine acting on H_2 receptors. The remaining component most likely involves a direct muscarinic action of acetylcholine on the parietal cells whereas gastrin does not play role (Yanagisawa 1990a; Tache 1994a) (Fig. 1). At submaximal effective doses of TRH injected intracisternally, however, the gastric acid response represents the net effect of released inhibitors such as prostaglandins and serotonin and stimulants such as histamine and acetylcholine interacting on the parietal cells (Ishikawa 1988; Yanagisawa 1990a; Yoneda 1992) (Fig. 1).

1.2.2.2 Motor Functions

There is a marked stimulation of gastric motor activity and gastric emptying upon central injection of TRH which is mediated by vagal muscarinic pathways (Garrick 1987; Madea-Hagiwara 1987; Tache 1990; Okumura 1995). Peripheral mechanisms of the motor response may also involve an interplay between the inhibitory (serotonin) and stimulatory (acetylcholine) influence of various neurotransmitters released (Fig. 1).

1.2.2.3 Gastric Mucosal Blood Flow

Intracisternal TRH or RX 77368 are well established to

increase gastric mucosal blood flow (GMBF) through vagal muscarinic pathways (Thiefin 1989; Tanaka 1993) . Such gastric hyperemia is independent of the increase in acid secretion since the hyperemic effect of central RX 77360 was not influenced by omeprazole-pretreatment (Thiefin 1989). The increase in GMBF is mainly mediated through nitric oxide pathways (Tanaka 1993).

1.2.2.4 Gastric mucosal resistance to acid (gastric mucus gel thickness, increased intraepithelial pH)

Intracisternal RX 77368 was shown to increase gastric mucus gel thickness, surface epithelial cell intracellular pH, HCO_3^- delivery and H^+ removal in the subepithelial interstitial space abutting the surface cells (Tache 1994a; Tanaka 1997). The mechanism through which central vagal activation increases gastric mucus gel thickness is independent from the prostaglandin secretion and probably mainly directly mediated by cholinergic pathways (Tache 1994a; Tanaka 1997).

1.2.2.5 Ulcer Formation and Protection

Recent studies have shown that intracisternal injection of TRH analog at low doses induces cytoprotection against gastric lesions induced by ethanol through vagal muscarinic release of prostaglandin E_2 (Madea-Hagiwara 1987; Yoneda 1992; Yoneda 1993b; Tache 1994b; Kaneko 1995a; Kaneko 1995b; Kato 1996). By contrast, central injection of TRH at maximal effective doses to stimulate gastric function leads to formation of gastric hemorrhagic lesions in fasted rats (Goto 1985; Tache 1988; Tache 1993b). Central sites of action have been located in the DVC and central amygdala (Tache 1994a).

Several data indicate that medullary TRH plays a role in cold restraint stress-induced gastric lesions. Central injection of TRH-antibody prevents gastric lesion formation induced by cold restraint (Yang 1994). Cold restraint induced an increase in TRH

gene expression and neuronal activation was observed as monitored by the appearance of c-fos immunoreactivity in nuclei raphe pallidus and obscurus (Yang 1994).

2. AIM AND OUTLINE OF THE THESIS

2.1. Studies on gastric cytoprotection

Pioneer findings by Robert et al established the unique ability of prostaglandins, in particular PGE_2, to prevent gastric mucosal injury elicited by a variety of experimental conditions independently of the changes in gastric acid secretion (Robert 1978).

The role of the parasympathetic nervous system in the development of gastric cytoprotection was investigated since:

(1.) either acute or chronic surgical vagotomy aggravates the gastric mucosal lesion formation in experimental ulcer models (Mozsik 1991; Mozsik 1992a; Kiraly 1992; Karadi 1994),

(2.) the activation of the vagus elicits gastric cytoprotection through prostaglandins (Yoneda 1992; Yoneda 1993b).

The aims of gastric cytoprotection studies were:

(1.) to investigate the action of TRH in the medulla to induce vagally-mediated gastric cytoprotection against ethanol,

(2.) to investigate the final peripheral mediators particularly the role of nitric oxide in the development of gastric cytoprotection induced by central vagal stimulation.

2.2 Vagal regulation of gastric functions: blood flow and acid secretion studies

The mechanisms involved in the vagal regulation of gastric mucosal blood flow were previously investigated by using electrical stimulation of the vagus nerve (Guth 1987). The associated stimulation of gastric acid secretion was originally postulated to account for the gastric hyperemic response (Guth

1987). However in vivo microscopy technique provided evidence for direct vasodilatory effect on gastric submucosal arterioles mediated by atropine-sensitive and atropine-resistant mechanisms observed at high frequencies of vagal stimulation (Morishita 1986; Guth 1987; Thiefin 1990). Since electrical stimulation of the vagal nerve trunk induced orthodromic as well as antidromic activation of vagal fibers (Thiefin 1990), injection into the cerebrospinal fluid of TRH or the stable analog, RX 77368, provides more relevant physiological tool to gain insight into the peripheral mechanisms underlying vagal-dependent stimulation of GMBF.

Although it is well established that central vagal activation produces hyperemia in the stomach, the peripheral mediators inducing the hyperemic effect are little known. Several vasoactive substances including acetylcholine, prostaglandins, histamine, VIP, nitric oxide, CGRP are candidates for peripheral mediator(s) of vasodilation during central vagal activation, since each of them were shown to be released after central injection of TRH or RX 77368 (Thiefin 1989; Lenz 1989; Yanagisawa 1990a; Thiefin 1990; Yoneda 1993b; Messmer 1993; Tanaka 1993).

The aims of gastric mucosal blood flow studies were to investigate:

(1.) the effect of intracisternal (ic) TRH analog, RX 77368 injected at maximal effective dose stimulating gastric acid secretion (30 ng) and at cytoprotective dose (1.5 ng), on the changes of GMBF, systemic blood pressure -mean arterial pressure (MAP)-, and gastric mucosal vascular resistance (GMVR) determined by a technique known to reflect only changes in gastric microcirculation (hydrogen gas clearance technique) (Leung 1984; Guth 1987; Livingston 1989),

(2.) the contribution of neuropeptides located in primary sensory neurons in the gastric submucosa to the increase in GMBF induced by intracisternal injection of maximal effective dose (30 ng) of TRH analog or peripheral cholinergic stimulation,

(3.) the nature of mediators participating in gastric hyperemia induced by ic injection of RX 77368 at 30 ng, to determine whether gastric hyperemia is muscarinic in nature or whether additional, atropine-resistant mechanisms also participate as observed with electric vagal stimulation (Guth 1987),

(4.) whether the atropine-dependent response is mediated by histamine and/or nitric oxide,

(5.) whether non-adrenergic-non-cholinergic neuropeptides such as vasoactive intestinal polypeptide (VIP) or tachykinins contribute in the action of central RX 77368 on the gastric microvasculature,

(6.) the effect of a cytoprotective (or threshold (O-Lee 1997)) dose (1.5 ng) of RX 77368 on gastric mucosal microcirculation and gastric acid secretion,

(7.) the role of prostaglandins and CGRP contained in capsaicin sensitive sensory afferent fibers in mediating or modulating GMBF and gastric acid secretory responses to intracisternal injection of RX 77368.

Furthermore we examined the antagonist action of recently synthesized vasoactive intestinal polypeptide (VIP) receptor antagonist, (4Cl-D-Phe^{8}, Leu^{17})VIP, on VIP-induced systemic and gastric vascular changes, and central vagal stimulation-induced gastric mucosal hyperemia and gastric acid secretion.

2.3 Investigation of the contribution of mucosal immune system in the effects of central vagal activation: brain-immune system axis

Cholinergic-mediated gastric mast cell degranulation has

been shown during stress (Cho 1979). Electric vagal stimulation was reported to induce an enhancement of mast cell activation in the rat stomach (Guth 1987). Furthermore, i.c. TRH analog increased the release of rat mast cell protease II (RMCPII) in the rat ileum through vagal muscarinic pathways (Saperas 1994). RMCPII is a specific marker for activated mucosal mast cells (MMC).

On the other hand consistent association of mast cells and nerve endings containing either substance P or CGRP was demonstrated in the skin, myocardium, ileum, mesentery and diaphragm (Stead 1987) of variety of animals.

Therefore the special aim of the studies were to asses the contribution of mucosal immune cells in the gastric mucosal hyperemia and gastric acid secretion induced by central vagal activation.

3. METHODS, RESULTS AND DISCUSSIONS

3.1 Studies on gastric cytoprotection

3.1.1 The role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation

3.1.1.1 Background

Central thyrotropin-releasing hormone (TRH) is well known to induce a vagal-dependent, muscarinic-mediated stimulation of different gastric functions in rats and cats (Tache 1988). Intracisternal (i.c.) injection of the stable TRH analog, RX 77368, was shown to prevent ethanol-induced gastric lesions in rats (Yoneda 1992; Tache 1994b; Kato 1994; Kaneko 1995a; Kato 1996). The cytoprotective effect involved muscarinic and prostaglandin mediated mechanisms (Yoneda 1993b; Tache 1994b; Kato 1996). Maintaining or increasing gastric mucosal blood flow plays an important role in gastric protection against injurious agents (Whittle 1990; Holzer, 1992b). TRH or RX 77368 injected i.c. stimulates gastric mucosal blood flow through muscarinic- and nitric oxide (NO)-dependent pathways (Thiefin 1989; Tanaka 1993). Since there are convergent evidence that NO is involved in the maintenance of the integrity of the gastric mucosa (MacNaughton 1989; Lopez-Belmonte 1992), the present study tests the hypothesis that the L-arginine-NO pathway plays a role in the gastric cytoprotection produced by i.c. injection of RX 77368 in rats.

3.1.1.2 Materials and methods

The following drugs were used: L-N^G-nitro-arginine methyl ester (L-NAME) and L- or D- arginine (Sigma Chemical, St Louis, MO), RX 77368 [p-Glu-His-(3,3'-dimethyl)-Pro-NH_2], (Reckitt & Colman, Kingston-upon-Hill, England), and ethanol (Fisher

Scientific, Fair Lawn, NJ). Drugs were diluted in 0.9% saline, except ethanol, which was diluted in distilled water. Injections into the cisterna magna or the jugular vein were given in volumes of 10 µl/rat and 0.3 ml/rat, respectively, under light ether anesthesia as described previously (Yoneda 1993b).

Male Sprague-Dawley albino rats (220-240 g, Harlan Laboratories, San Diego, CA) were fasted overnight but allowed water ad libitum up to the beginning of the experiments. Rats were injected i.v. with saline or L-NAME (3 mg/kg) immediately before i.c. injection of saline or RX 77368 (1.5 ng). Additional groups of rats were injected i.v. with either L- or D-arginine (500 mg/kg) or saline immediately before L-NAME. In a separate experiment, groups of rats were injected i.v. with L-arginine (100-500 mg/kg) or D-arginine (500 mg/kg). Ethanol (60%, 1 ml) was administered intragastrically to conscious rats 30 min after i.c. or i.v. injections. The choice of doses for each treatment was based on our previous studies (Yoneda 1992; Yoneda 1993b; Tanaka 1993). Rats were killed by CO_2 inhalation 60 min after ethanol administration, and stomachs were removed, opened along the greater curvature, gently rinsed in 0.9% saline, and pinned open to expose the mucosa. The percentage of corpus mucosa containing lesions was determined by means of a computerized image analyzer device (MICRO/PDP-11, Digital Equipment Corp., Maynard MA), equipped with imaging boards (Imaging Technology Inc., Woburn MA) as described previously (Yoneda 1992).

Results are expressed as means ± S.E.M. A one way analysis of variance (ANOVA) followed by Duncan's contrast were used to compare the differences between groups. P value < 0.05 was considered to be statistically significant.

3.1.1.3 Results

Oral administration of 1 ml of 60% ethanol to rats injected i.c. with saline produced macroscopic lesions covering 10.7 ± 1.8% of the total surface of the corpus mucosa (Fig. 2). L-NAME

alone did not increase significantly the extent of mucosa damage induced by ethanol (13.8 ± 1.8%). RX 77368 injected i.c. at 1.5 ng 30 min before ethanol instillation reduced by 88% the extent of gastric mucosa injury (1.3 ± 0.3%). The gastric cytoprotection induced by RX 77368 was completely abolished by i.v. injection of 3 mg/kg L-NAME (11.9 ± 1.9%). The effect of L-NAME was antagonized by i.v. injection of 500 mg/kg of L-arginine (3.5 ± 1.4%) but not by D-arginine (10.3 ± 3.3%) (Fig. 2). The highest dose of L-arginine (500 mg/kg) alone protected the gastric mucosa against ethanol (2.7% ± 0.8%) whereas the lower doses (100 and 300 mg/kg) and D-arginine (500 mg/kg) were ineffective (Fig. 3).

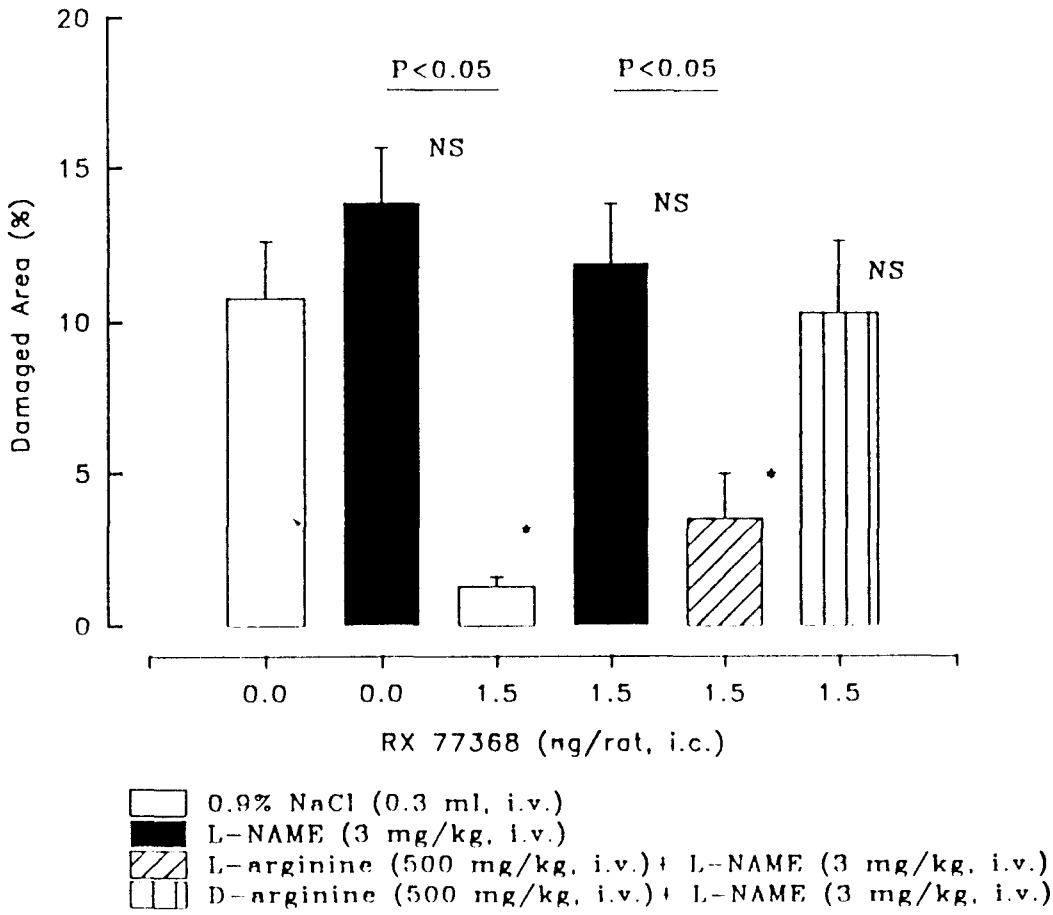

Fig. 2. Effect of l-NAME on i.c. RX 77368-induced gastric cytoprotection in ethanol-treated rats. Treatments (vehicle:0.9% NaCl: 0.3 ml iv, white columns; l-NAME: 3 mg/kg iv, black column; l-arginine: 500 mg/kg iv +l-NAME: 3 mg/kg iv, oblique strip column; D-arginine: 500 mg/kg iv + l-NAME: 3 mg/kg iv, vertical strip column) were given 30 min before intragastric administration of ethanol (60%, 1 ml). Each column represents the mean ± S.E.M. of 7-11 rats per group. * P< 0.05 vs. i.c. saline plus ethanol.

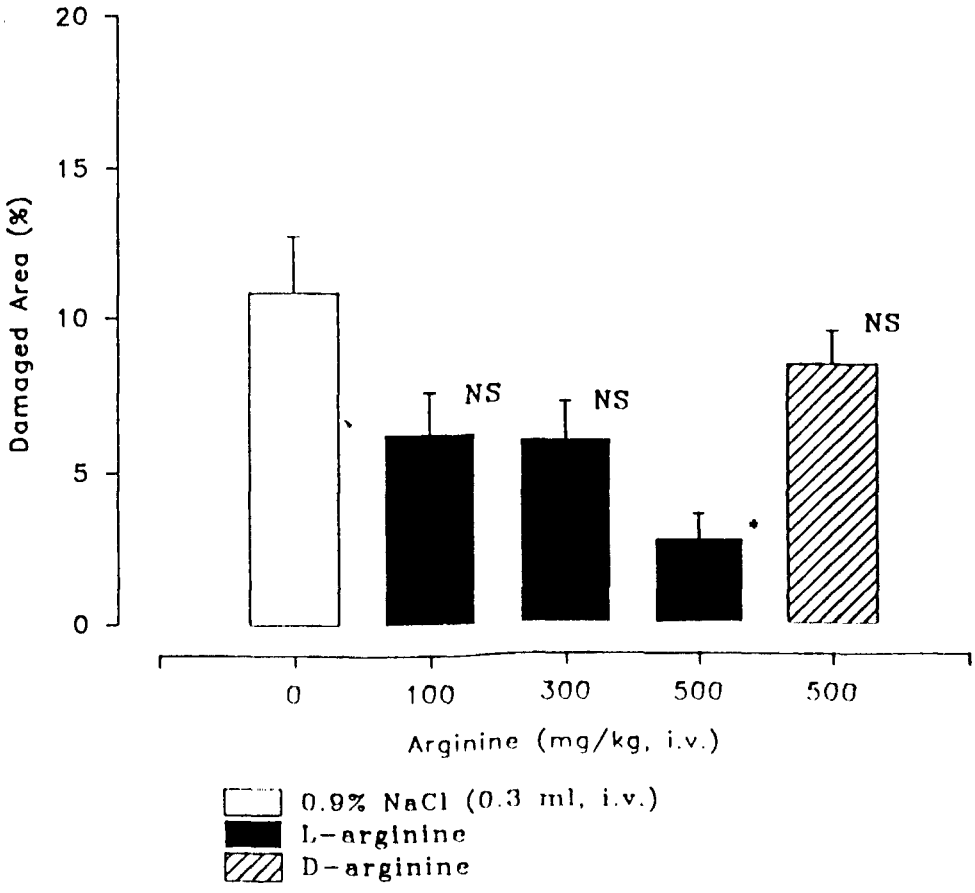

Fig. 3. Effects of L- or D-arginine on the development of ethanol-induced gastric mucosa lesions. Vehicle (0.9% NaCl: 0.3 ml iv, white column), L-arginine (100, 300 or 500 mg/kg iv, black columns) and D-arginine (500 mg/kg iv, strip column) were given 30 min before ig ethanol (60%, 1 ml). Each column represents the mean ± S.E.M * P< 0.05 vs. ethanol alone, n=6-8 per group.

3.1.1.4 Discussion

In agreement with our previous report, i.c. injection of a low dose of TRH analog RX 77368 protects the gastric mucosa against ethanol-induced damage (Yoneda 1992). The cytoprotective

effect of i.c. injection of RX 77368 was shown to be mediated through central vagal cholinergic pathways (Yoneda 1992). In the present study, L-NAME, injected i.v. at a dose that has previously been established to inhibit NO synthesis from L-arginine (MacNaughton 1989; Tanaka 1993), did not influence ethanol-induced gastric lesions while it completely abolished the cytoprotective effect of RX 77368. The action of L-NAME was reversed in an enantiomerically specific manner by L- but not D-arginine. These results indicate a role for the L-arginine NO pathway in mediating the cytoprotective effect of centrally injected TRH analog. This is further supported by evidence that central vagal activation by i.c. RX 77368 increases gastric NO formation (Tanaka 1993; Tanaka 1997). In addition, i.v. injection of the NO precursor, L-arginine, prevents ethanol-induced gastric lesions whereas the D-arginine, which does not induce NO synthesis, had no effect. L-arginine given i.v. is also reported to provide protection against HCl-induced gastric damage (Tanaka 1997) and other NO donors to reduce gastric lesions induced by endothelin-1 and ethanol in an ex vivo chamber preparation of the rat stomach. In addition, there is evidence that inhibition of NO synthesis prevents intragastric capsaicin- and calcitonin-gene related peptide-induced protection against ethanol (Lambrecht 1993). Taken together, previous and present findings provide convergent evidence for a role of NO in the protection of the gastric mucosa against injury.

The mechanisms through which NO mediates i.c. RX 77368-induced cytoprotection probably involve an action on the microvasculature. NO is a potent vasodilator (Palmer 1988) and the maintenance of gastric microcirculation is a key element in the resistance to ethanol injury (Whittle 1990). In addition, we previously demonstrated that i.c. RX 77368 increases gastric mucosal blood flow through muscarinic NO-dependent pathways (Thiefin 1989; Tanaka 1993). Since we previously found that prostaglandins and calcitonin gene-related peptide also participate in the cytoprotective effect of i.c. RX 77368 (Yoneda

1993b; Kato 1994; Tache 1994b; Kato 1996), the demonstration of an involvement of NO pathways further supports the concept that an interaction exists between NO, prostanoids and sensory peptide in the regulation of mucosal integrity as reported by Whittle et al. (Whittle 1990).

3.2 Vagal regulation of gastric functions: blood flow and acid secretion studies

3.2.1 Central vagal activation by TRH induces gastric hyperemia: role of CGRP in capsaicin-sensitive afferents in rats

3.2.1.1 Background

TRH or its stable analog, RX 77368, injected into the cisterna magna or the dorsal vagal complex, enhances the efferent activity of the gastric branch of the vagus nerve leading to vagal stimulation of gastric secretory and motor function (Somiya 1984; Garrick 1987; Madea-Hagiwara 1987; Tache 1988; Tache 1990; Tache 1993a; Chan 1995). Central injection of TRH also increases gastric mucosal blood flow (GMBF) through vagal dependent pathways (Okuma 1987; Ishikawa 1988; Thiefin 1989; Raybould 1990; Tanaka 1993). Electrical vagal stimulation increases GMBF through cholinergic and non cholinergic components (Morishita 1986; Thiefin 1990) . However, the increase in GMBF in response to central injection of TRH is solely mediated by vagal cholinergic dependent mechanisms (Thiefin 1989). It has been reported previously that the gastric hyperemic response to intracisternal injection of TRH or TRH analog is not a secondary event to the vagal stimulation of gastric acid secretion (Thiefin 1989), but represents a direct vasodilatory response mediated by muscarinic and nitric oxide dependent mechanisms (Thiefin 1989; Tanaka 1993).

In the rat skin microvasculature, acetylcholine-induced vasodilation involves both nitric oxide and capsaicin-sensitive components (Ralevic 1992). Anatomical and functional observations are consistent with the possibility that the gastric hyperemia resulting from central vagal cholinergic activation may also involve the recruitment of capsaicin-sensitive afferent fibers. First, capsaicin-sensitive primary sensory neurons, which

contain α-calcitonin-gene related peptide, innervate the vasculature and form a dense plexus around arterioles and small arteries of the mucosa and submucosa in the rat stomach (Sternini, 1992). Second, α-CGRP is well established as one of the most potent stimulants of GMBF in rats (Holzer 1991a). Lastly, recent reports indicate that the gastric vasodilatory and cytoprotective effects of α-CGRP involve nitric oxide dependent pathways (Holzer 1993; Lambrecht 1993).

In the present study, we assessed the contribution of CGRP located in primary sensory neurons to the increase in GMBF induced by intracisternal injection of TRH or peripheral cholinergic stimulation.

3.2.1.2 Materials and methods

Animal Preparations

Male Sprague-Dawley rats (Harlan Laboratories, San Diego, CA) weighing 250-275 g were housed under conditions of controlled temperature (20± 3°C) and illumination (12-h light cycle starting at 6 AM). Rats were maintained on Purina Laboratory Rat Chow (Ralston Purina, St. Louis, MO) and tap water *ad libitum*. The food was withheld for 24 h before the experiment, but water was given ad libitum. All experiments were performed in rats anesthetized with urethane (1.25 g/kg i.p.). Rectal temperature was maintained between 36-37°C throughout the duration of experiments with heat lamps.

Drugs and Treatments

The following substances were used: the stable TRH analog, RX 77368, [p-Glu-His-(3,3'-dimethyl)-Pro-NH_2; Reckitt & Colman, Kingston-upon-Hill, England]; the CGRP receptor antagonist, human $CGRP_{8-37}$, [$hCGRP_{8-37}$; Institute National de Recherche en Santé, University of Québec, Pointe-Claire, P.Q., Canada]; rat α-CGRP [Clayton Foundation Laboratories, The Salk Institute, La Jolla,

CA]; substance P (NK_1) receptor antagonist, CP-96,345, [Pfizer Inc., Groton, CT]; and bethanechol chloride [Merck Sharp & Dohme, West Point, PA]. All other chemicals were obtained from Sigma Chemical Co., (St. Louis, MO). RX 77368 was aliquoted in 0.1% bovine serum albumin (BSA) containing 0.9% saline at a concentration of 3 μg/10 μl and kept frozen at -20° C. The stock solution was dissolved in 0.9% saline (pH=7.0) before administration. $CGRP_{8-37}$ and CP-96,345 were dissolved in 0.1% BSA containing 0.9% saline. Capsaicin was dissolved in absolute ethanol, Tween 80 and isotonic saline, 10:10:80:v/v/v. In rats under light ether anesthesia, capsaicin was injected s.c. three times at 12 h intervals at doses of 25, 50 and 50 mg/kg, respectively. The control group received the same regimen of injections except that the vehicle instead of capsaicin was administered. Experiments were performed 10-14 days after completion of the vehicle or capsaicin pretreatment in rats that showed the disappearance of the corneal chemosensory reflex to a drop of a 0.1% NH_4OH instillated into both eyes.

Surgical Procedures

Placement of catheter into the cisterna magna.

The head was fixed in a stereotaxic instrument (Kopf model 900) and the atlanto-occipital membrane was exposed. With the help of a dissecting microscope, a small pin hole was made into the membrane with a 25 G needle at 1-1.5 mm distal to the caudal edge of the occipital bone. Then a PE-10 polyethylene catheter (length: 10 cm; dead space: ca 5 μl) was inserted through the hole into the cisterna magna. The catheter was connected to a 50 μl Hamilton microliter syringe. Successful cannulation was verified by leakage of cerebrospinal fluid (CSF) from the catheter. A drop of cyanoacrylate (Crazy Glue, Itasca, Ill) was used to hold the catheter in position. Intracisternal injection of RX 77368, (30 ng in 10 μl volume) was followed by a 10 μl injection of saline to flush the catheter.

Tracheal cannula and other vessels.

After cannulation of the cisterna magna, a tracheotomy was performed to facilitate ventilation and enable the administration of hydrogen and the esophagus was ligated. A PE-50 catheter was placed into the femoral artery for monitoring blood pressure with a Statham P23 Dd transducer. Another PE-50 catheter was inserted into the jugular vein to deliver drugs either for bolus injection (0.3 ml over 30 sec) and infusion (1.5 ml/h) to maintain hydration. Close intra-arterial infusion was performed by inserting retrogradely a PE-10 catheter into the splenic artery close to the celiac artery. The infusion volume into the splenic artery was 25 μl/min.

Gastric cannula.

To avoid the backdiffusion of gastric acid secretion stimulated after RX 77368 injection, the stomach was cannulated as follows. After a midline laparotomy, the stomach was exteriorized, the pylorus was ligated and an incision was made in the forestomach to insert a double-lumen cannula (outer Tygon tube and inner polyethylene catheter 7 mm and inner 2 mm diameter, respectively) which was secured by ligature. Physiological saline at room temperature was infused by the inner cannula at a rate of 0.5-0.7 ml/min. The effluent was continuously collected by flow drainage from the outer tube.

Position of electrode.

After a small incision on the surface of the anterior wall of the stomach, a platinum needle electrode was inserted from the serosa into the basal portion of the gastric mucosa. The electrode was positioned in a high blood flow area, between the two branches of the left gastric artery, closer to the lesser than the greater curvature of the corpus of the stomach as previously described (Raybould 1990; Tanaka 1993). The reference electrode (Ag-AgCl) was placed inside of the peritoneal cavity.

Measurement of GMBF and Vascular Resistance.

After an equilibration period of 1 h, GMBF was measured by the hydrogen gas-clearance technique as described previously (Leung 1984). Each measurement involved a 30 min period including 15 min of saturation with 3% hydrogen gas and 15 min desaturation of the tissue. The gas clearance was analyzed by a computerized monoexponential direct curve-fitting program (Livingston 1989). Value of GMBF are expressed in ml/min/100 g. The mean arterial blood pressure (MAP) was recorded continuously throughout the duration of each experiment and expressed in mm Hg. Gastric mucosal vascular resistance (GMVR) was calculated by dividing the MAP taken at the beginning of the desaturation period by the respective blood flow and expressed in mm Hg/ml/min/100 g. The MAP time point was selected since the first minutes after the end of saturation period, the desaturation is rapid and has a maximal influence on the final curve fit measuring GMBF.

Experimental Protocols

Study 1. Effect of i.v. administration of CGRP receptor antagonist on i.v. α-CGRP-induced gastric hyperemia.

The inhibitory effect the CGRP antagonist, $hCGRP_{8-37}$ against α-CGRP-induced increase in GMBF was first tested under our experimental conditions. GMBF was measured twice before and twice after starting the administration of vehicle (0.1% BSA containing 0.9% saline, i.v., n=5) or $hCGRP_{8-37}$ (15 μg/kg i.v. bolus, followed by an infusion of 3 μg/kg/h throughout the experiment, n=5). Then, both groups were infused with α-CGRP (14 μg/kg/h) into the splenic artery for a 30 min period during which GMBF was measured. The choice of the dose of α-CGRP was based on a previous study showing that close intra-arterial infusion of the peptide stimulates GMBF in urethane-anesthetized rats (Holzer 1991a). The dose of CGRP antagonist was based on preliminary

studies.

Study 2. Effect of the CGRP receptor antagonist, $hCGRP_{8-37}$, or substance-P antagonist, CP-96,345, given i.v. on intracisternal RX 77368-induced gastric hyperemia.

In the second experiment, GMBF was measured twice before and twice after $hCGRP_{8-37}$ (14 μg/kg, i.v. bolus injection followed by 3 μg/kg/h, n=6) or vehicle (0.1% BSA containing 0.9% NaCl, i.v., n=7) then, RX 77368 (30 ng) was injected intracisternally to both groups. Changes in GMBF were measured at 30 min intervals for 3 h starting 5 min after TRH analog injection. In another group of rats, after two basal GMBF measurements, vehicle or the substance P receptor antagonist, CP-96,345 (3 mg/kg) was injected as a bolus. Five min later vehicle or RX 77368 (30 ng) was injected intracisternally and after 5 min, the 30 min GMBF measurement was performed. The dose of CP-96,345 injected i.v. was based on previous studies showing the inhibition of substance P-induced salivation for over 90 min in rats (Plourde 1993; Snider 1991). The dose of TRH analog (30 ng) was selected based on previous studies showing a robust stimulation of GMBF under these conditions (Tanaka 1993).

Study 3. Effect of capsaicin pretreatment on i.v. CGRP antagonist-induced inhibition of gastric hyperemic response to intracisternal RX 77368.

In capsaicin- or vehicle-pretreated rats, GMBF was measured twice before and twice after administration of vehicle or $hCGRP_{8-37}$ (15 μg/kg i.v. bolus, followed by an infusion of 3 μg/kg/h throughout the experiment). Then, RX 77368 (30 ng) was injected intracisternally to all groups (n=4 per group) and 5 min later, the 30 min period measurement of GMBF was performed over the 3 h experimental period.

Study 4. Influence of i.v. CGRP antagonist on bethanechol-induced gastric hyperemia.

GMBF was measured twice before and twice after the administration of vehicle or $hCGRP_{8-37}$ (15 μg/kg i.v. bolus, followed by an infusion of 3 μg/kg/h throughout the experiment); then bethanechol chloride (150 μg/kg/h) was infused close-arterially to both groups (n=4 per group) and GMBF was measured. The dose of bethanechol was selected to reproduce a similar increase in GMBF as that induced by intracisternal injection of TRH analog (30 ng).

Statistics

Results are expressed as means ± SEM. Comparisons between two groups were calculated by Student's t-test, multiple group comparisons were performed by ANOVA followed by Duncan's contrast. $P<0.05$ was considered as statistically significant.

3.2.1.3 Results

Study 1. Effect of i.v. administration of CGRP receptor antagonist on i.v. α-CGRP-induced gastric hyperemia.

Close intra-arterial infusion of α-CGRP (14 μg/kg/h) for 30 min increased significantly GMBF (ml/min/100 g) from basal level of 41.4 ± 4.4 to 72.9 ± 11.2 and decreased significantly MAP (mm Hg) and GMVR (mm Hg/ml/min/100 g) from 77 ± 3 to 57 ± 6 and 1.9 ± 0.2 to 0.8 ± 0.1 respectively (Fig. 4A-C). Systemic infusion of $hCGRP_{8-37}$ (15 μg/kg i.v. bolus followed by an infusion of 3 μg/kg/h) did not influence vascular parameters and completely prevented the α-CGRP-induced elevation of GMBF and declines in MAP and GMVR (Fig. 4A-C).

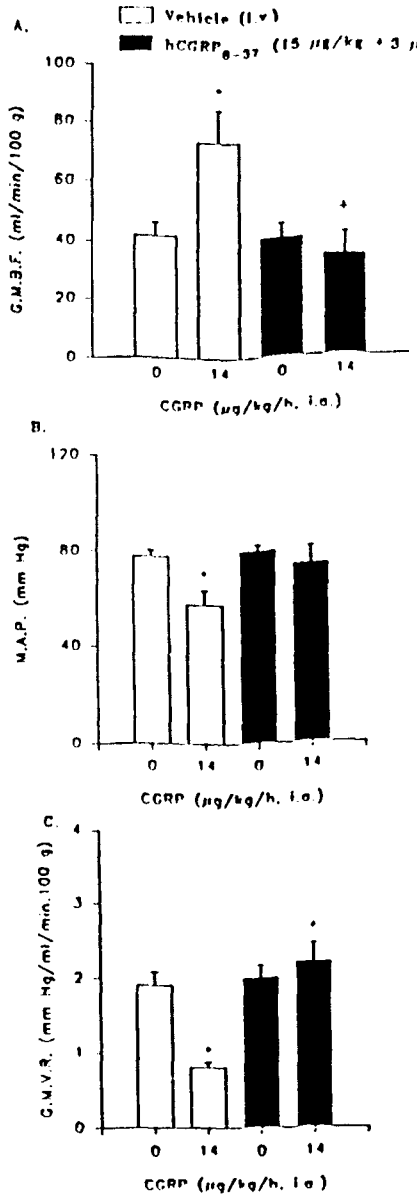

Fig. 4. Influence of the CGRP receptor antagonist, $hCGRP_{8-37}$ on close intra-arterial infusion of α-CGRP induced vascular changes in urethane-anesthetized rats. After two basal measurements, $hCGRP_{8-37}$ (black columns) or vehicle (white columns) was injected intravenously (iv) as a bolus (15 µg/kg or 0.3 ml) then as an intravenous infusion (3 µg/kg/h or 1.5 ml/h) throughout the experiment. α-CGRP (14 µg/kg/h) was infused intra-arterially (ia) for 30 min to both groups, starting at 60 min after the bolus injection. Each column represents the mean ± SEM of 5 rats. Measurements represent the 30-60 min period after the beginning of the infusion of vehicle or CGRP antagonist alone and the first 30 min period after α-CGRP infusion with vehicle or CGRP antagonist. * $P<0.01$ compared with vehicle alone; + $P<0.01$ compared with vehicle plus α-CGRP treated group.

Study 2. Effect of the CGRP receptor antagonist, $hCGRP_{8-37}$, or substance-P antagonist, CP-96,345, given i.v. on intracisternal RX 77368-induced gastric hyperemia.

Before treatment, the two experimental groups had similar basal GMBF (ml/min/100 g: 53.5 ± 3.6 and 55.3 ± 8.1), MAP (mm Hg: 76 ± 3 and 77 ± 2) and GMVR (mm Hg/ml/min/100 g: 1.6 ± 0.2 and 1.7 ± 0.2) (Fig. 5A-C). Neither $hCGRP_{8-37}$ nor vehicle administered for 1 h, influenced basal GMBF, MAP, and GMVR values (Fig. 5A-C). In the vehicle-pretreated group, intracisternal injection of RX 77368 (30 ng, i.c.) elevated GMBF, which reached a peak (123.6 ± 16.1 ml/min/100 g) during the first 30 min period post injection (Fig. 5A). A significant elevation was still observed during the following 30 min period and thereafter values returned to the basal levels (Fig. 5A). $hCGRP_{8-37}$ infusion abolished RX 77368-induced increase in GMBF (Fig. 5A). In vehicle- or $hCGRP_{8-37}$-infused groups, intracisternal injection of RX 77368 elevated MAP values that reached 116 ± 6 mm Hg and 127 ± 4 mm Hg respectively. Thereafter, MAP values declined similarly in both groups to return to basal levels at the third 30 min period after TRH analog injection (Fig. 5B). Intracisternal injection of RX 77368 decreased GMVR in vehicle-treated animals and increased GMVR in the CGRP antagonist-treated group during the first two 30 min period after TRH analog injection (Fig. 5C). No gastric erosions were observed by visual inspection after intracisternal injection of RX 77368 under these conditions.

Intravenous injection of the substance P receptor antagonist, CP-96,345 (3 mg/kg), modified neither the basal GMBF, MAP and GMVR levels nor RX 77368-induced rises of GMBF and MAP and reduction of GMVR (Table 1).

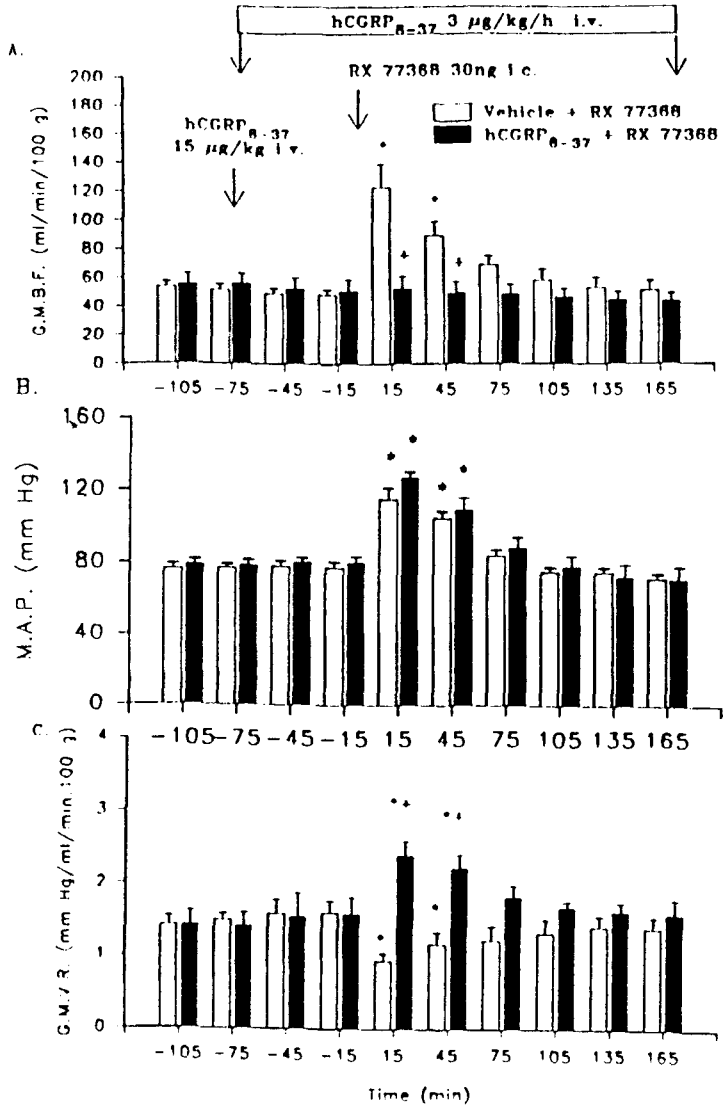

Fig. 5. Influence of the CGRP receptor antagonist, $hCGRP_{8-37}$ (15 µg/kg bolus + 3 µg/kg/h iv, black columns) or vehicle (0.3 ml + 1.5 ml/h, iv, white columns) on RX 77368-induced stimulation of gastric mucosal blood flow (A), mean arterial pressure (B) and gastric mucosal vascular resistance (C) in urethane-anesthetized rats. Each column represents the mean ± SEM of 6-7 rats/group. * P<0.05 compared with respective basal values; + P<0.05 compared with vehicle plus RX 77368-treated group.

Table 1. Effect of the substance P antagonist, CP-96,345, on intracisternal TRH analog-induced increases in gastric mucosal blood flow (GMBF) in urethane-anesthetized rats.

Treatment[a]	N	GMBF[b] (ml/min/100 g)	MAP[b] (mm Hg)	GMVR[b] (mmHg/ml/min/100 g)
Basal	12	55.3±8.1	77± 8	1.4±0.4
Veh.+Veh.	4	51.3±8.1	76±5	1.5±0.1
CP-96,345 + Vehicle	4	51.9±3.6	78±6	1.5±0.2
CP-96,345 + RX 77368	4	128.0±24.0*	106±7*	0.8±0.1*

[a] After a basal GMBF measurement, vehicle or CP-96,345 was injected (3 mg/kg, i.v.), then 5 min later, vehicle or TRH analog, RX 77368 (30 ng) was given intracisternally. GMBF measurement was performed during the next 30 min period. MAP was monitored through the femoral artery at 15 min before injection of vehicle or CP-96,345 (basal), and 15 min after intracisternal injection.

[b] Mean ± SEM of number of rats listed as N, * $P<0.05$ compared with basal values.

Study 3. Effect of capsaicin pretreatment on i.v. CGRP antagonist-induced inhibition of gastric hyperemic response to intracisternal RX 77368.

Sensory nerve ablation by systemic capsaicin pretreatment did not alter the basal GMBF (58.3 ± 5.8 ml/min/100 g), MAP (84 ± 2 mm Hg), and GMVR (1.4 ± 0.1 mm Hg/ml/min/100 g) (Fig. 6A-C) compared with vehicle-pretreated rats (GMBF: 55.3 ± 7.9 ml/min/100 g; MAP: 80 ± 8 mm Hg, GMVR: 1.4 ± 0.2 mm Hg/ml/min/100 g) (Fig. 5A-C). Intravenous administration of $hCGRP_{8-37}$ did not modify basal GMBF, MAP and GMVR values in both vehicle- or capsaicin-pretreated rats (Fig. 6A-C). The CGRP antagonist, $hCGRP_{8-37}$ completely prevented the rise of GMBF induced by RX 77368 in vehicle-pretreated rats (Fig. 6A) as observed in non vehicle-pretreated rats (Fig. 5A). However, intravenous administration of $hCGRP_{8-37}$ failed to reverse the gastric hyperemic response to RX 77368 (30 ng, i.c.) in capsaicinized rats (Fig. 6A). In capsaicin-pretreated rats, the elevation of GMBF observed during the first 30 min period after RX 77368 injection reached its peak: 135.5 ± 18.6 ml/min/100 g, then values decreased to basal level within 90 min after the injection. The magnitude and duration of the gastric hyperemic response to intracisternal injection of RX 77368 in capsaicin-pretreated rats were similar to those observed in non pretreated rats (Figs. 5A and 6A). RX-77368 induced a similar rise in MAP in vehicle- and capsaicin-pretreated rats infused either with vehicle or the CGRP antagonist (Fig. 6B). $hCGRP_{8-37}$ did not block the decrease in GMVR induced by RX 77368 in capsaicin-pretreated rats (Fig. 6C).

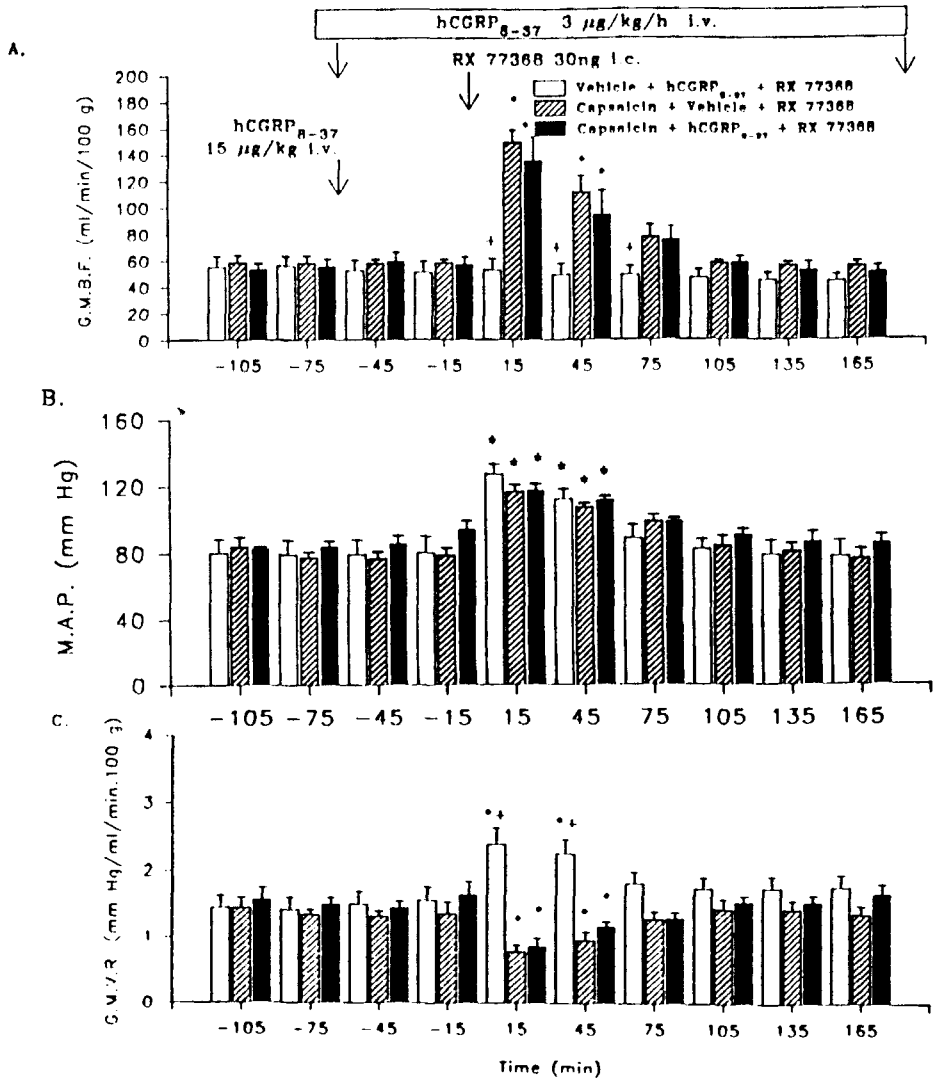

Fig. 6. Influence of CGRP receptor antagonist, $hCGRP_{8-37}$ (15 µg/kg + 3 µg/kg/h, iv) on intracisternal injection of RX 77368-induced stimulation of gastric mucosal blood flow (A), mean arterial pressure (B) and gastric mucosal vascular resistance (C) in capsaicin- (strip or black columns) or vehicle- pretreated (white columns), urethane-anesthetized rats. Each column represents mean ± SEM of 4 rats/group. * $P<0.05$ compared with respective basal values; + $P<0.05$ compared with vehicle-pretreated group.

Study 4. Influence of i.v. CGRP antagonist on bethanechol-induced gastric hyperemia.

Close intra-arterial infusion of bethanechol (150 μg/kg/h) enhanced the basal GMBF from 52.4 ± 5.1 ml/min/100 g to 106 ± 15.8 ml/min/100 g (Fig. 7A) with a parallel decrease in GMVR from 1.7 ± 0.2 to 0.7 ± 0.1 mm Hg/ml/min/100 g during the first 30 min period of infusion (Fig. 7C). Systemic administration of $hCGRP_{8-37}$ under the same conditions as in previous studies 1-3 inhibited the vascular responses to bethanechol (GMBF: 67.7 ± 8.7 ml/min/100 g, GMVR: 1.4 ± 0.2 mm Hg/ml/min/100 g). The MAP was not altered by close arterial infusion of bethanechol in vehicle- or CGRP antagonist-pretreated rats (Fig. 7B).

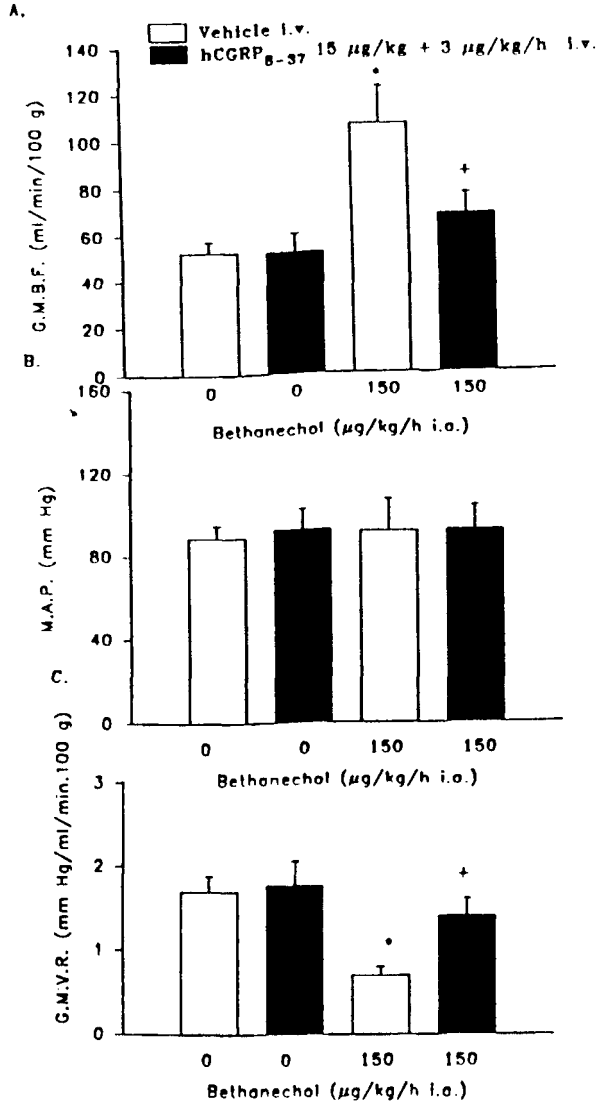

Fig. 7. Influence of CGRP receptor antagonist, $hCGRP_{8-37}$ (15 μg/kg + 3 μg/kg/h, iv) on close arterial infusion of bethanechol-induced (150 μg/kg/h, ia) stimulation of gastric mucosal blood flow (A), mean arterial pressure (B) and gastric mucosal vascular resistance (C) in urethane-anesthetized rats. MAP was monitored through the femoral artery at 15 min before (basal) and 15 min after i.v. administration of drugs. Each column represents the mean ± SEM of 4 rats/group. * $P<0.05$ compared with respective basal values; + $P<0.05$ compared with vehicle plus bethanechol-treated group.

3.2.1.4 Discussion

Intracisternal injection of the stable TRH analog, RX 77368 at 30 ng increased the GMBF by 131% during the first 30 min period after the injection as measured by the hydrogen gas clearance technique in urethane-anesthetized rats. Thereafter, GMBF values are decreasing to reach basal levels within 90 min after peptide injection. Likewise, previous reports indicate that RX 77368 injected into the cisterna magna at 30 ng or TRH injected into the lateral ventricle or dorsal vagal complex induced a robust increase in GMBF assessed by the hydrogen gas- or aminopyrine-clearance technique (Okuma 1987; Thiefin 1989; Raybould 1990; Tanaka 1993) .

Convergent evidence demonstrated that the gastric hyperemic response to central injection of TRH or RX 77368 is mediated by vagal efferent cholinergic pathways.

First, the increase in GMBF can be reproduced by direct microinjection of TRH into the dorsal motor nucleus of the vagus (Okuma 1987).

Second, injection of TRH or RX 77368 into the CSF stimulates efferent activity recorded in the cervical or the gastric branch of the vagus (Somiya 1984; Mattila 1986).

Third, vagotomy completely abolished the rise in GMBF induced by TRH injected centrally (Thiefin 1989).

Fourth, in the present study, the muscarinic agonist, bethanechol, infused close intra-arterially to the stomach at a dose which did not influence systemic blood pressure, elevates GMBF by 102% but decreases vascular resistance by 41%.

Fifth, peripheral but not central injection of the muscarinic antagonist, atropine, completely suppressed the increase in GMBF induced by central injection of TRH (Okuma 1987; Thiefin 1989).

Our previous studies indicate that RX 77368 injected intracisternally at 30 ng induced a maximal acid secretory response 30 min post injection in urethane-anesthetized rats (Raybould 1990; Tanaka 1993). However, the rise in GMBF induced

by TRH injected into the CSF is not secondary to vagal cholinergic stimulation of acid secretion since the hyperemic response was maintained in omeprazole-treated rats (Thiefin 1989). The increase in GMBF and decrease in gastric vascular resistance is also not related to the alterations in MAP. Injection of TRH or RX 77368 into the CSF increased MAP (Forster 1993b) and present observation which is indicative of an increase in total systemic vascular resistance, while in the gastric submucosa, vascular resistance was found to be decreased (Tanaka 1993), present observation. Convergent electrophysiologic and functional evidence have established that the increase in MAP and heart rate induced by TRH injected into the CSF results solely from sympathetic hyperactivity. By contrast, the gastric mucosal hyperemia originates mainly from activation of vagal cholinergic pathways (Mattila 1986; Thiefin 1989). These data along with the existing knowledge on the organization of the gastric microcirculation (Guth 1987) indicate that the increase in GMBF must result from vagal muscarinic mediated vasodilation occurring locally in the gastric submucosal arterioles.

Several peptide exert a vasodilatory effect, however CGRP was established to be one of the most potent vasodilators of blood vessels in the gastric mucosa and submucosa as well as in other vascular beds. The peptide affects only resistance (arteries and arterioles) and not capacitance vessels (veins and venules) (Brain 1985; Holzer 1991a; Chen 1992). In the present study, two specific observations indicate that CGRP participates in the rise in GMBF induced by intracisternal injection of RX 77368 in intact rats.

First, consistent with several previous studies, α-CGRP infused intra-arterially close to the stomach increased GMBF while the vascular resistance and MAP were lowered in urethane-anesthetized rats (Holzer 1991a; Chen 1992). The enhanced GMBF despite reduced perfusion pressure reflects effective dilation of gastric mucosal blood vessels in agreement with previous findings (Holzer 1991a). The use of in vivo microscopy and topical

application of α-CGRP on gastric submucosal arterioles further confirmed that α-CGRP exerts a direct vasodilatatory action on gastric submucosal arterioles involving interaction with CGRP-1 receptor subtype (Chen 1992). Likewise, in the present study, the systemic and gastric vascular effects of close intra-arterial infusion of α-CGRP were completely prevented by intravenous infusion of the CGRP-1 receptor antagonist, $hCGRP_{8-37}$. The CGRP-1 receptor mediated vasodilatory effect of α-CGRP is well correlated with the dense representation of α-CGRP binding sites in the arteries and arterioles of the gastric submucosa (Gates 1989).

Second, when $hCGRP_{8-37}$ was infused at a dose blocking the gastric hyperemic response to exogenous α-CGRP and having no effect on basal GMBF, the 131% rise in GMBF induced by intracisternal injection of the TRH analog was completely inhibited. Likewise, the CGRP antagonist reduced by 73% the similar increase in GMBF induced by bethanechol infused close intra-arterially to the stomach. Topical application of bethanechol was reported previously to induce an atropine-sensitive dilatation of gastric submucosal arterioles (Morishita 1986). The decrease in vascular resistance induced by intracisternal injection of RX 77368 and by close arterial infusion of bethanechol was also counteracted by the infusion of $hCGRP_{8-37}$. By contrast, the rise in MAP induced by central TRH analog was not altered by the CGRP antagonist. These data further show that intracisternal injection of the TRH analog increases GMBF independently from changes in systemic blood pressure. CGRP was previously reported to play a major role in the gastric hyperemic response induced by acute exposure of the mucosa to capsaicin and acid back diffusion (Li 1991; Li 1992). However, the present findings provide the first evidence to suggest that CGRP participates in the peripheral mechanisms responsible for the gastric hyperemia induced by central vagal cholinergic activation.

Immunohistochemical studies demonstrate that α-CGRP in the rat stomach is contained almost exclusively in unmyelinated capsaicin-sensitive afferent neurons originating mainly from the dorsal root ganglia (Raybould 1992; Sternini, 1992). Systemic capsaicin pretreatment ablates the dense CGRP immunoreactivity contained in splanchnic neurons around blood vessels in the gastric mucosa (Raybould 1992; Sternini, 1992). In addition, functional evidence indicates that capsaicin-sensitive sensory neurons play an important role in the regulation of GMBF mainly through the local release of CGRP from peripheral terminals (Holzer, 1988; Chen 1992; Raybould 1992). In the present study, $hCGRP_{8-37}$ no longer inhibits the 157% increase in GMBF induced by intracisternal injection of RX 77368 in capsaicin-pretreated rats. By contrast, in animals pretreated with vehicle, the CGRP antagonist blocked the rise in GMBF as observed in non pretreated rats. These findings indicate that $hCGRP_{8-37}$ antagonizes the biological action of CGRP originating primarily from capsaicin-sensitive afferents. These data suggest that in intact rats central vagal activation induces CGRP released from capsaicin-sensitive afferent terminals which exerts a local "efferent" function on GMBF (Holzer, 1988).

Increase in GMBF is one of the major mechanism through which capsaicin-sensitive afferents mediate the prevention of gastric mucosal injury induced by ethanol (Holzer 1991b). We previously reported that intracisternal TRH or RX 77368 at low doses induced protection against ethanol-induced gastric lesions (Yoneda 1992; Yoneda 1993b; Kiraly 1993). Such gastric cytoprotective action of central TRH was also shown to involve CGRP recruited from capsaicin-sensitive sensory neurons (Kato 1994). The present observations may have implications to the understanding of CGRP dependent mechanisms mediating the gastric cytoprotection induced by intracisternal injection of TRH analog.

Although the small mesenteric arterioles of the submucosa of the stomach are densely innervated by CGRP containing fibers

(Sternini, 1992), substance P is also present to a lesser extent in capsaicin-sensitive spinal afferent fibers (Sharley 1984). However, previous studies indicate that substance P does not stimulate GMBF (Gepetti 1991; Holzer 1991a; Gronbech 1994). Likewise, in the present study, injection of CP-96,345 a selective substance P antagonist, at the neurokinin-1 receptor subtypes (Snider 1991) was ineffective in altering the GMBF in response to intracisternal injection of the TRH analog. The antagonist was given at a dose established to prevent the actions of substance P in other biological systems (Snider 1991; Plourde 1993). These data further demonstrated the specificity of CGRP antagonist action on the gastric hyperemic response to RX 77368 injected intracisternally.

The mechanisms through which the stimulation of central vagal muscarinic efferent pathways activate the local "effector" functions of C-terminal capsaicin-sensitive neurons containing CGRP (Holzer, 1988) remain to be investigated. Acid back diffusion-induced gastric mucosal hyperemia is mediated by CGRP release from capsaicin- sensitive fibers (Gepetti 1991; Li 1992). Gastric acid secreted in response to injection of RX 77368 was removed from the stomach by continuous perfusion with 0.9% NaCl, pH=7. In addition intracisternal injection of TRH analog at the 30 ng dose not induce gastric mucosal lesions within 30 min. Therefore it is unlikely that the activation of CGRP release from capsaicin-sensitive afferent fibers is secondary to changes in gastric acid secretion or mucosal integrity. Possible mechanisms may involve a direct excitatory action of acetylcholine on peripheral nociceptive C-fibers as recently demonstrated in the vasodilatory response to acetylcholine in the rat skin microvasculature (Ralevic 1992). We previously demonstrated that intracisternal injection of RX 77368 induced a vagal cholinergic mediated release of PGE_2 (Yoneda 1993b), histamine (Yanagisawa 1990a), and serotonin (Stephens 1989; Yang 1992). Prostaglandins and serotonin have been shown to sensitize

the majority of the C-polymodal afferents and to activate capsaicin-sensitive afferent C-fibers leading to the release of CGRP in the rat trachea and lungs (Lundberg 1992; Hua 1993). Furthermore histamine, and serotonin were reported to sensitize and/or to excite certain limited populations of capsaicin-sensitive polymodal C-fiber afferents in the lung, or stomach (Lundberg 1992; Hua 1993). It can be hypothesized that these agents activate sensory C-fibers that lead to the release of CGRP in the gastric mucosa.

Vagal cholinergic release of nitric oxide was recently reported to be involved in mediating the gastric hyperemic and cytoprotective effects induced by intracisternal injection of TRH analog (Tanaka 1993). The involvement of nitric-oxide dependent pathways may be rèlated to α-CGRP receptor activation. Nitric oxide is the major secondary mediator of the gastric hyperemic and protective actions of CGRP released from sensory nerve endings in the stomach (Holzer 1993; Lambrecht 1993). The complete abolition of the gastric hyperemic response to intracisternal injection of RX 77368 at 30 ng by either the CGRP antagonist (present observation) or by the nitric oxide synthase inhibitor, L-NAME, (Tanaka 1993) is compatible with such a CGRP-dependent nitric oxide activation resulting in gastric vasodilation in intact animals.

In capsaicin-pretreated rats in which gastric sensory afferents containing CGRP are ablated, intracisternal injection of RX 77368 still increased GMBF with a similar magnitude as in vehicle-pretreated rats. Further studies indicate that the nitric oxide synthase inhibitor, L-NAME, abolished intracisternal RX 77368 (30 ng)-induced rise in GMBF in capsaicin-pretreated rats (Kiraly 1997b). In addition, L-NAME action was reversed by L- but not by D-arginine (Kiraly 1997b). These findings suggest that in capsaicin-pretreated rats, nitric oxide increases GMBF through vagal efferent cholinergic mechanisms independently from CGRP pathways as demonstrated in the skin vasculature (Ralevic 1992).

In summary, these findings suggest that in capsaicin-treated rat, central vagal activation induced by TRH analog increases GMBF by CGRP independent mechanisms. By contrast, in intact rats, the gastric hyperemia induced by intracisternal injection of TRH analog at 30 ng dose involves vagal cholinergic activation of the local "effector function" of capsaicin-sensitive fibers releasing the vasodilatory peptide CGRP. These data provide evidence that upon central vagal activation, there is an interaction between vagal efferent cholinergic pathways and sensory afferent fibers containing CGRP. Such cross talk may have functional implications as activated capsaicin sensory afferents may generate local as well as central reflex modulating gastric response to vagal efferent stimulation.

3.2.2 Peripheral mediators involved in gastric hyperemic response to vagal activation by central TRH analog in rats

3.2.2.1 Background

Central injection of TRH or TRH analogs stimulate gastric vagal efferent discharges (Somiya 1984; O-Lee 1997) and gastrointestinal secretory and motor functions and modulate the resistance of the gastric mucosa to injury through vagal atropine sensitive pathways (Tache 1988; Tache 1994a) . Central TRH also stimulates exocrine pancreatic and duodenal bicarbonate secretion and net ileal and jejunal water secretion through vagal atropine-sensitive as well as insensitive pathways (Lenz 1989; Messner 1993; Lenz 1995). The physiological role of medullary TRH was further established by the demonstration that TRH antibody microinjected into the cisterna magna or DMN abolished the vagally mediated adaptive gastric protection, and gastric responses to cold exposure, 2-deoxy-D-glucose and chemical activation of midline raphe cell bodies (Yang 1993; Kaneko 1995a; Kaneko 1995b; Okumura 1995).

The mechanisms involved in the vagal regulation of gastric mucosal blood flow (GMBF) were previously investigated using electrical stimulation of the vagus nerve (Guth 1975; Guth 1987). The associated stimulation of gastric acid secretion was originally postulated to account for the gastric hyperemic response (Guth 1987). However, in vivo microscopy techniques provided evidence for a direct vasodilatory effect on gastric submucosal arterioles mediated by atropine-sensitive (cholinergic) and atropine resistant mechanisms particularly at high frequencies of vagal stimulation (Guth 1987; Thiefin 1990). Since electrical activation of the vagal nerve trunk can produce orthodromic as well as antidromic activation of vagal fibers (Thiefin 1990), injection into the cerebrospinal fluid of TRH or the stable analog, RX 77368 provides a more relevant

physiological tool to gain insight into the peripheral mechanisms underlying changes in GMBF induced by vagal efferent activation (Thiefin 1989; Tanaka 1993; Kiraly 1994).

In earlier studies, we have shown that central injection of TRH or RX 77368 increased GMBF independently from the stimulation of gastric acid secretion and prostaglandin generation in urethan-anesthetized rats (Thiefin 1989; Tanaka 1993; Tanaka 1997). In particular, RX 77368 injected ic at 1.5 ng activated gastric vagal efferent discharge (O-Lee 1997) and increased GMBF while being subthreshold to stimulate gastric acid secretion (Yoneda 1993b; Kiraly 1997b). The hyperemic response under these conditions was primarily mediated by an "efferent function" of capsaicin-sensitive primary afferent fibers containing calcitonin gene-related peptide (CGRP) (Kiraly 1997b). By contrast, when RX 77368 was injected at 30 ng, leading to a robust stimulation of gastric vagal efferent activity and acid secretion (Tanaka 1993; O-Lee 1997), the increase in GMBF was not altered by capsaicin (-10 days) alone or in combination with intravenous injection of CGRP antagonist or omeprazole at a dose blocking the stimulated acid secretion (Kiraly 1994; Tanaka 1997). The vasodilator transmitters involved under conditions of sustained central vagal efferent stimulation in capsaicin-pretreated rats have not yet been identified. Therefore, the present study was undertaken to explore the mediators participating in the regulation of GMBF after ic injection of RX 77368 at 30 ng in urethan-anesthetized rats. We determined whether gastric hyperemia is muscarinic in nature or whether additional, atropine resistant, mechanisms also participate, as observed with electrical vagal stimulation at high frequency (Guth 1987; Thiefin 1990). Secondly, we investigated whether the atropine-dependent response is mediated by histamine, and/or nitric oxide (NO) using the histamine receptor antagonist (H_1-), pyrilamine, and L-NMMA (N^G-nitro-monomethyl-L-arginine), a NO synthase inhibitor which is devoid of direct muscarinic antagonist properties (Buxton 1993). These

substances are potential candidates as they are released or generated through vagal muscarinic dependent mechanisms in the gastric effluent after ic injection of RX 77368 at 30 ng (Yanagisawa 1990a; Yoneda 1993b; Saperas 1995) and have vasodilatory properties in the gastric mucosa (Guth, 1991; Guth 1987; Whittle, 1993). We also investigated whether the GMBF response maintained in capsaicin-pretreated rats after ic injection of RX 77368 at 30 ng (Kiraly 1994) is sensitive to NO synthase inhibition. Lastly, in view of the report that intravenous injection of L-NAME inhibits NO synthase activity in the rat forebrain (iadecola 1994), we also compared the effects of peripheral (iv) vs central (ic) injection of L-NAME (N^G-nitro-L-arginine-methylester) to ascertain the peripheral action of NO inhibitors.

3.2.2.2 Materials and methods

Drugs

The following substances were used: the stable TRH analog, RX 77368 (p-Glu-His-(3,3'-dimethyl)-Pro-NH_2) (Reckitt & Colman, Kingston-upon-Hill, England), atropine sulfate, capsaicin, pyrilamine, L-NAME (N_G-nitro-L-arginine-methylester), L-NMMA (N_G-nitro-monomethyl-L-arginine), L-arginine hydrochloride and D-arginine hydrochloride, all from Sigma Chemical Co. (St. Louis, MO). RX 77368 was aliquoted in 0.1% bovine serum albumin (BSA) and 0.9% saline at a concentration of 3 μg/10 μl, and kept frozen at -70 °C. The stock solution of RX 77368, and atropine sulphate, pyrilamine, L-NAME, and L-NMMA in powder form were dissolved in 0.9% saline (PH=7.0) before administration. Capsaicin was dissolved in vehicle (absolute ethanol, Tween 80 and isotonic saline, 10:10:80:v/v/v). Unless otherwise stated, the volume for ip, sc, and iv bolus injections was 0.3 ml and iv infusion was 1.5 ml/h.

Animal Preparations and Surgical Procedures

All the surgical procedures performed for simultaneous measurements of GMBF and mean arterial blood pressure (MAP) and for drug administration were essentially as previously described in chapter 3.2.

Experimental Protocols

In all experiments, after one or two basal GMBF measurements, pretreatment was given and, at different time intervals thereafter, the TRH analog was injected ic at 30 ng. GMBF and MAP measurements were performed at various time intervals before and after ic injection. When pretreatments were injected ip or sc, saline 1.5 ml/h was infused to maintain hydration.

Study 1. Effect of atropine, and pyrilamine.

In the first series of experiments, after basal GMBF and MAP measurements, rats received vehicle (0.9% saline, sc) or atropine sulfate (2 mg/kg, sc); pyrilamine (1 mg/kg iv bolus followed by 2 mg/kg/h, iv infusion) or vehicle (0.9% saline, 0.3 ml iv bolus followed by 1.5 ml/h iv infusion). Thirty min after atropine and 60 min after pyrilamine, RX 77368 (30 ng) was injected ic and GMBF, MAP and GMVR were monitored before and up to 120 min after RX 77368 injection.

Study 2. Effect of capsaicin, L-NMMA and L-NAME.

After basal measurements, L-NMMA (50 mg/kg, iv) or vehicle (0.9% saline, iv) was administered and 15 min later, RX 77368 (30 ng) was injected ic. The selection of L-NMMA dose was based on an equal rise in blood pressure as L-NAME (Rees 1990). In a second set of studies, rats were injected sc with capsaicin (25, 50 and 50 mg/kg in 0.5 ml) at 12-h intervals. The first capsaicin injection was performed under short enflurane anesthesia. After 10-14 days, capsaicin-pretreated rats showed the disappearance of the corneal chemosensory reflex (eye wiping for 1-3 min to a drop

of 0.1% NH_4OH instillated into each eye when tested just before the experiment). After basal GMBF measurements, capsaicin-pretreated rats were injected iv with vehicle (saline), L-NAME (10 mg/kg), L-NAME (10 mg/kg) plus L-arginine (500 mg/kg) or L-NAME (10 mg/kg) plus D-arginine (500 mg/kg) and 15 min later, RX 77368 (30 ng) was injected ic. GMBF, MAP and GMVR were measured before and after ic injection of RX 77368 (30 ng). In the third set of experiments, after basal measurements, L-NAME (500 μg/kg, ic) or vehicle (saline, ic) was injected 15 min before RX 77368 (30 ng, ic) or vehicle (saline). Each substance was injected ic in a 5 μl volume followed by 5 μl flush of the catheter with saline (total volume: 20 μl). The changes in GMBF, MAP and GMVR were monitored before and after RX 77368.

Statistics

Results are expressed as means ± SEM. Comparisons between two groups were calculated by Student's t-test. Comparisons before and after treatment were analyzed by Student's paired t-test. Multiple group comparisons were performed by ANOVA followed by Duncan's contrast. $P<0.05$ was considered statistically significant.

3.2.2.3 Results

Study 1. Effect of atropine and pyrilamine on GMBF, MAP and GMVR Changes Induced by Intracisternal RX 77368 at 30 ng

The basal GMBF was 54.2 ± 8.0 ml/min/100 g (Fig. 8A), MAP 80.4 ± 6.9 mmHg (Fig. 8B), and vascular resistance, 1.6 ± 0.3 mm Hg/ml/min/100 g (Fig. 8C) in urethan-anesthetized rats. GMBF, MAP and GMVR remained stable when measured again at a 30 min interval and following sc injection of either vehicle or atropine (Fig. 8A-C). In the vehicle-injected group, RX 77368 (30 ng, ic) evoked a peak increase in GMBF during the first period measurement after peptide injection reaching 138.9 ± 16.2 ml/min/100 g which

represents a 157% increase from pre-injection levels (Fig. 8A). Blood pressure also significantly increased to 116 ± 3 mm Hg at 15 min after ic injection of TRH while GMVR significantly decreased to 0.9 ± 0.1 mmHg/ml/min/100 g (Fig. 8B,C). Thereafter, changes in GMBF, MAP and GMVR declined and reached preinjection basal values at 75 min post injection (Fig. 8). Atropine (2 mg/kg, sc, -30 min) completely abolished RX 77368 (30 ng, ic)-induced stimulation of GMBF and decrease in GMVR while the rise in MAP was not significantly modified (Fig. 8). Pyrilamine infusion (1 mg/kg bolus + 2 mg/kg/h, iv) influenced neither the basal nor RX 77368-induced increase in GMBF and MAP and decrease in GMVR (Table 2).

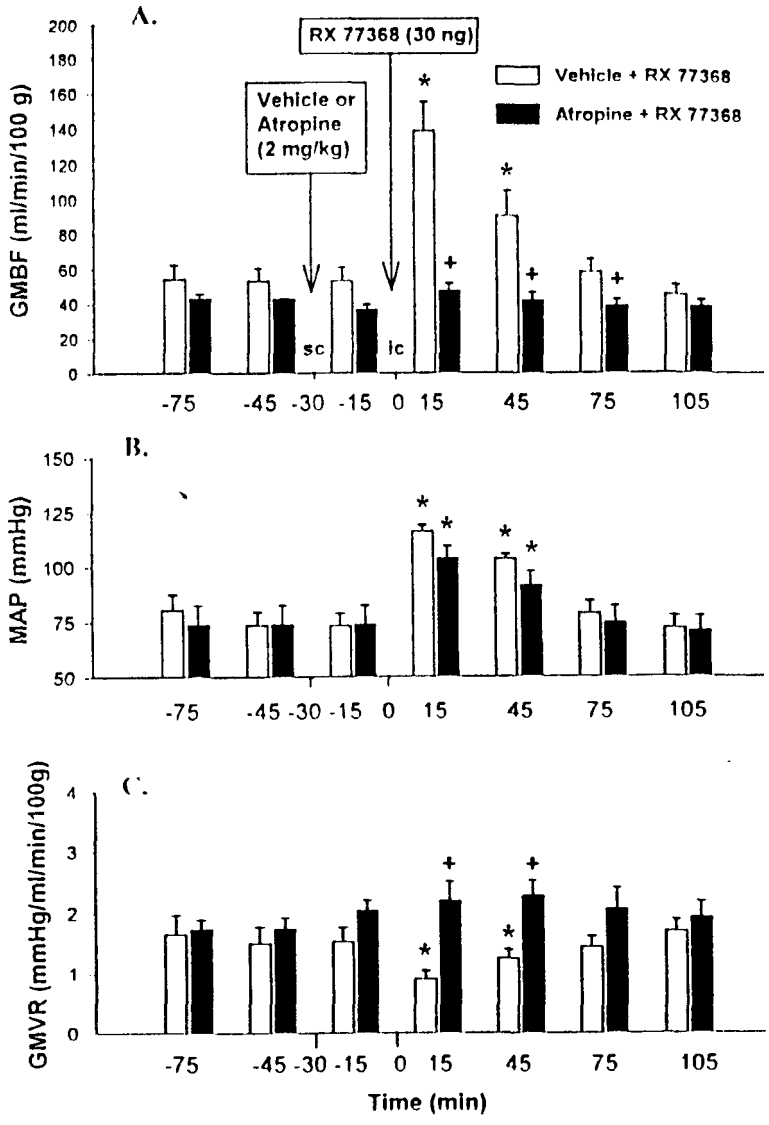

Fig. 8. Influence of atropine (2 mg/kg, sc) on RX 77368-induced changes in gastric mucosal blood flow (GMBF), mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) in urethan-anesthetized rats. Each column represents mean ± SEM of 4-5 rats/group. * P< 0.05 compared with respective basal values; + P< 0.05 compared with vehicle plus RX 77368-treated group.

Table 2. Effect of pyrilamine on intracisternal RX 77368-induced changes of GMBF, MAP and GMVR in urethane-anesthetized rats

Treatments[a]	N	GMBF (ml/min/100 g)[b]	MAP (mmHg)[b]	GMVR (mmHg/ml/min/100 g)[b]
Basal	11	51.0±3.0	78±3	1.6±0.1
Vehicle	7	48.7±3.5	76±5	1.6±0.2
Vehicle + RX 77368	7	123.5±16.1*	114±3*	0.9±0.1*
Pyrilamine	4	50.8±8.0	82±3	1.7±0.3
Pyrilamine + RX 77368	4	125.4±33.2*	118±6*	0.9±0.4*

[a] After basal measurements, vehicle (0.3 ml + 1.5 ml/h, iv), or pyrilamine (1 mg/kg bolus + 2 mg/kg/h iv) was injected. After an additional 45 min, GMBF, MAP and GMVR were measured and 15 min later, RX 77368 (30 ng, ic) was injected. Measurement was performed during the next 15-30 min period.

[b] Mean ± SEM of number of rats shown in column n. *: $P < 0.05$ compared with basal.

Study 2. Effect of L-NMMA, capsaicin alone or with L-NAME

Intravenous injection of L-NMMA (50 mg/kg) completely abolished the increase in GMBF elicited by ic injection of RX 77368 (30 ng) (Fig. 9A). The rise in blood pressure induced by ic injection of RX 77368 was further enhanced by L-NMMA in magnitude (+20 mmHg) and duration (105 min instead of 75 min to reach basal level) (Fig. 9B). Vascular resistance which was decreased by ic RX 77368 in vehicle-treated rats was enhanced in the presence of L-NMMA compared with preinjection values for the 75 min period post injection (Fig. 9C).

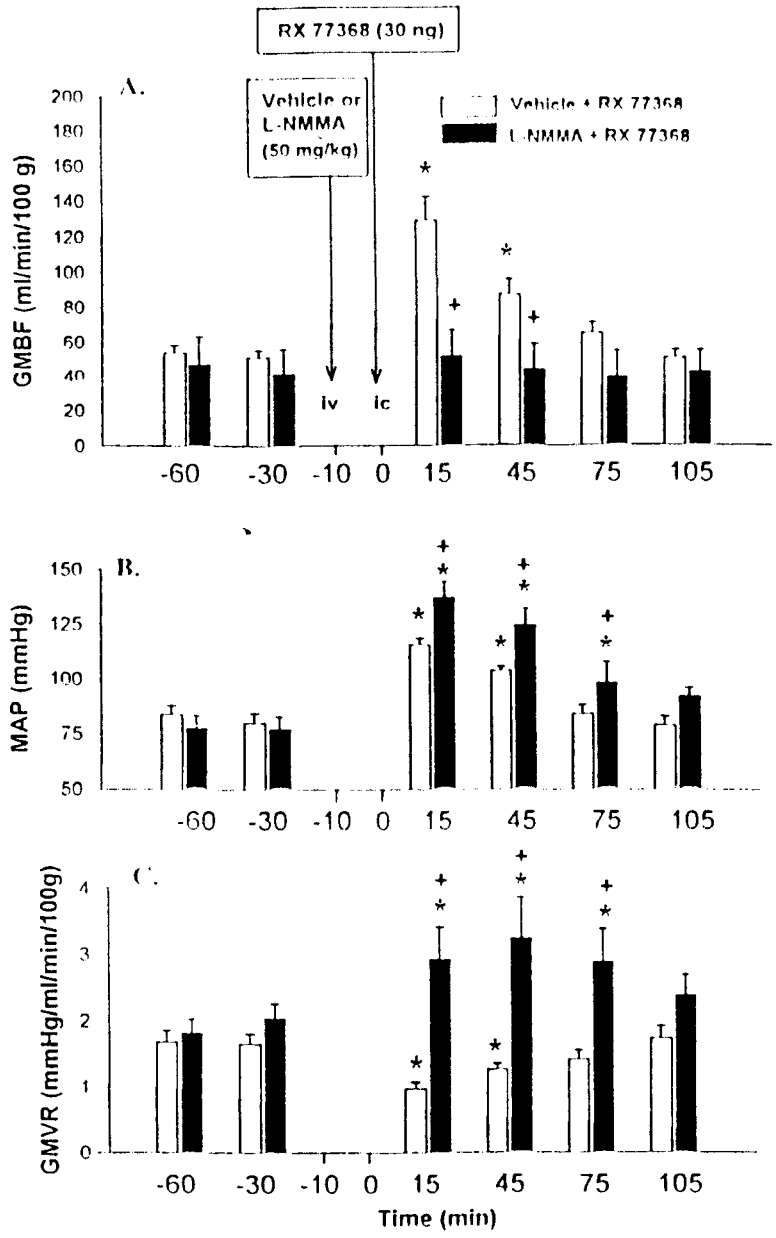

Fig. 9. Influence of NG-monomethyl-l-arginine (l-NMMA) (50 mg/kg, iv, black columns) or vehicle (0.3 ml, iv, white columns) on RX 77368-induced changes in gastric mucosal blood flow (GMBF), mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) in urethan-anesthetized rats. Each column represents mean ± SEM of 4-9 rats/group. * $P < 0.05$ compared with respective basal values; + $P < 0.05$ compared with vehicle plus RX 77368-treated.

In capsaicin-pretreated rats, ic injection of RX 77368 (30 ng) induced a peak in the increase of GMBF (ml/min/100 g) from 58.2 ± 2.9 to 149.5 ± 9.5 and decreased GMVR during the 15-30 min post injection, then values returned to preinjection levels (Fig. 10A,C). Pretreatment with L-NAME (10 mg/kg, -15 min) completely abolished the increase in GMBF and decrease in GMVR induced by RX 77368 (Fig. 10A,C). L-arginine (500 mg/kg) injected iv before L-NAME restored the increase in GMBF and decrease in GMVR induced by ic TRH analog. However, under the same conditions of iv administration, the stereoisomer, D-arginine (500 mg/kg), failed to reverse the inhibitory effect of L-NAME on gastric vascular changes induced by ic TRH analog (Fig. 10A,C). In L-NAME or L-NAME plus D-arginine treated groups, injected ic with RX 77368, GMVR was increased compared with respective pre-injection values (Fig. 10C). The rise in MAP induced by ic injection of RX 77368 in capsaicin-pretreated rats was further enhanced by L-NAME in magnitude (+ 27 mmHg) and duration (over 105 min) (Fig. 10B). L-arginine, unlike D- arginine, reversed the amplifying effect of L-NAME on blood pressure while not influencing the rise induced by ic RX 77368 (Fig. 10B).

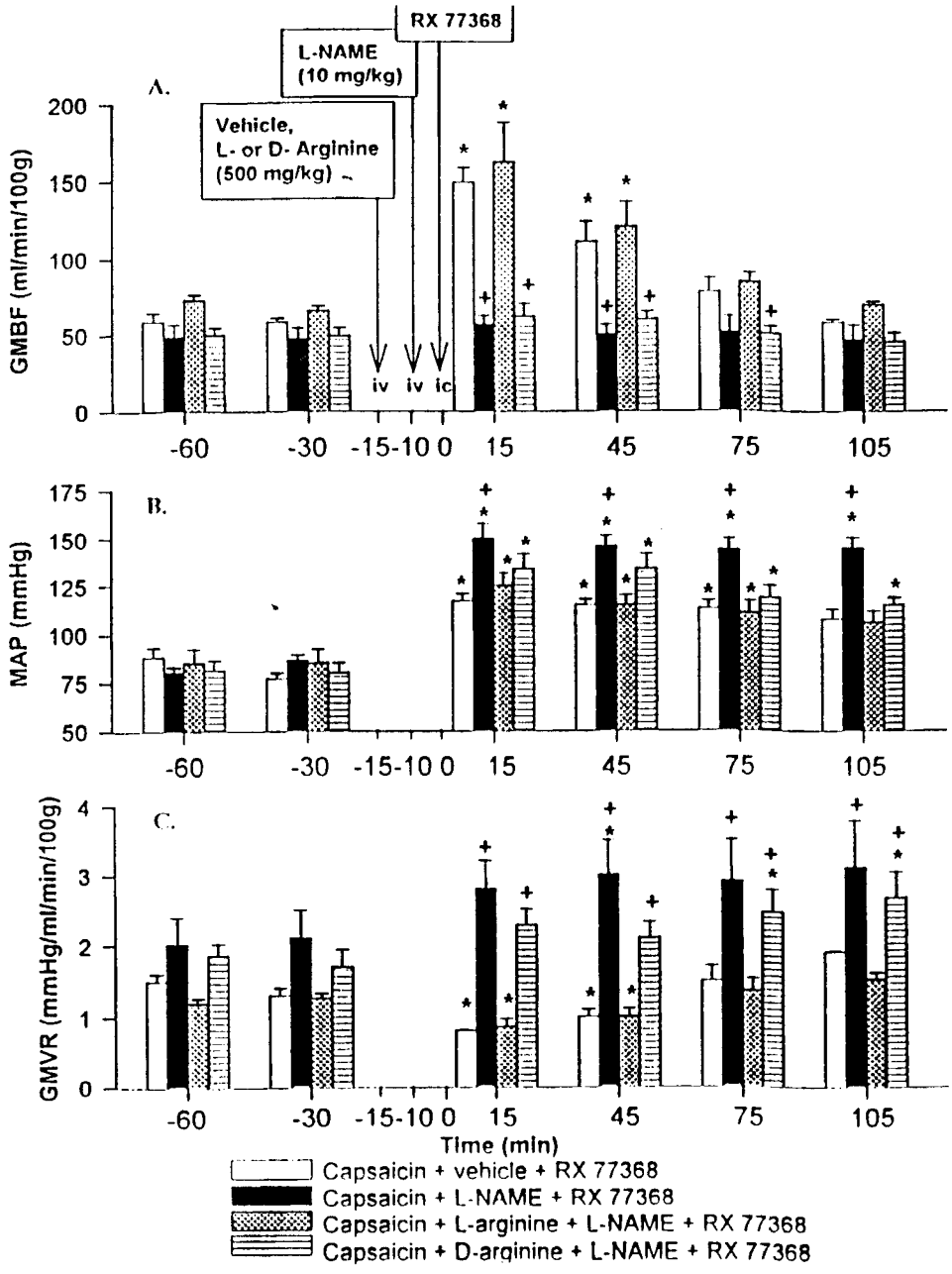

Fig. 10. Influence of NG-nitro-L-arginine methyl ester (L- NAME) alone (10 mg/kg, iv, black columns) or combined by L-or D-arginine (500 mg/kg, iv, scattered or stripped columns, respectively) on RX 77368-induced changes in gastric mucosal blood flow (GMBF), mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) in urethan-anesthetized rats pretreated with capsaicin. Each column represents mean ± SEM of 4-9 rats/group. * P< 0.05 compared with respective basal values; + P< 0.05 compared with vehicle plus RX 77368-treated

L-NAME (500 μg/kg) injected into the cisterna magna did not alter ic injection of TRH analog (30 ng) induced changes in GMBF, GMVR and MAP (Table 3). L-NAME injected ic alone did not influence basal GMBF but increased MAP and GMVR (Table 3).

Table 3. Effect of ic injection of L-NAME on ic RX 77368-induced changes in GMBF, MAP, GMVR in urethan-anesthetized rats

Treatments[a]	N	GMBF (ml/min/100 g)[b]	MAP (mm Hg)[b]	GMVR (mm Hg/ml/100 g)[b]
Basal	20	49.0±2.9	81±3	1.7±0.1
Veh. + Veh.	4	50.8±6.4	77±6	1.6±0.2
Vehicle + RX 77368	4	123.0±15.2*	114±3*	1.0±0.2*
L-NAME + Vehicle	4	52.5±1.4	117±5*	2.2±0.2*
L-NAME + RX 77368	8	114.7±9.9*	114±5*	1.1±0.1*

[a]After basal GMBF and GMVR measurements, vehicle (5 μl, ic) or L-NAME (500 μg/kg, ic) was injected. Fifteen min later, vehicle or RX 77368 (30 ng, ic) was injected and GMBF and GMVR measurements were performed during the next 30 min period. MAP was monitored through the femoral artery, 30 min before (basal) the beginning of ic injections and 15 min after the administration of RX 77368.

[b]Mean ± SEM of number of rats indicated in column N. *: $P < 0.05$ compared with vehicle + vehicle group.

3.2.2.4 Discussion

The stable TRH analog, RX 77368 injected ic at 30 ng increased GMBF as shown by the hydrogen gas clearance technique in urethan-anesthetized rats. These results are consistent with previous studies using intracerebroventricular injection of TRH at 1-5 μg or intracisternal administration of RX 77368 at a similar or lower dose (1.5 ng) and monitoring the increase in GMBF by either the aminopyrine clearance, laser Doppler or hydrogen gas clearance techniques (Thiefin 1989; Tanaka 1993; Kiraly 1994; Tanaka 1997). The gastric hyperemic response to central TRH or RX 77368 is mediated by vagal dependent pathways.

First, injection of TRH (3-300 ng) or RX 77368 (1.5-15 ng) resulted in a 90-145% and 140-244% increase in the gastric vagal efferent discharges respectively in urethan-anesthetized rats (O-Lee 1997).

Second, selective microinjection of TRH into the dorsal motor nucleus of the vagus mimicked the effect of peptide injection into the cerebrospinal fluid (Okuma 1987).

Third, vagotomy completely abolished the increase in GMBF evoked by TRH injected intracerebroventricularly (Thiefin 1989).

Convergent sets of evidence in rats indicate that central vagal activation-induced an increase in GMBF through an atropine-sensitive mechanism. In the present study, atropine completely inhibited the 3 fold increase in GMBF elicited by ic RX 77368 at 30 ng. Likewise, atropine completely blocked intracerebroventricular injection of TRH-induced 2 fold stimulation of GMBF in urethan-anesthetized rats (Thiefin 1989). In dogs, atropine also blocked the increase in blood flow induced by vagal stimulation by 2-deoxy-D-glucose or insulin (Swan 1967). In addition, acetylcholine infused intra-arterially into the stomach increases GMBF and acetylcholine applied topically onto gastric submucosal arterioles induces

vasodilatation through atropine-sensitive mechanisms (Morishita 1986; Kitagawa 1987a). By contrast, upon electrical vagal activation, gastric blood flow response involves an additional atropine-resistant component (Kitagawa 1987a; Thiefin 1990). Capsaicin sensitive vagal afferents (Thiefin 1990) and/or postganglionic release of non-adrenergic non-cholinergic vasodilators such as vasoactive intestinal peptide have been postulated to contribute to the atropine-resistant component of gastric hyperemia to electrical vagal activation (Ito 1988). Since medullary TRH plays a physiological role in the vagal efferent regulation of gastric function (Tache 1994a), it is tempting to speculate based on the present and previous data that under activation of medullary preganglionic vagal neurons, the underlying mechanisms increasing GMBF are mainly atropine-sensitive.

We examined whether the rise in GMBF resulted from a direct effect of acetylcholine or was secondary to a muscarinic mediated release of vasodilatory substances. RX 77368 injected ic stimulated the gastric generation of PGE_2 and histamine release and activated the efferent function of capsaicin sensitive afferents through atropine-sensitive pathways (Yanagisawa 1990a; Yanagisawa 1990b; Yoneda 1993b). These transmitters contribute to the modulation of gastric acid secretion and resistance of the mucosa to injury elicited by ic RX 77368 (Yanagisawa 1990a; Yanagisawa 1990b; Yoneda 1993b; Tache 1993b). PGE_2 and histamine, acting preferentially through H_1 receptors as well as CGRP contained in capsaicin sensitive afferents increase GMBF in rats (Guth 1987; Guth, 1991; Holm 1992; Holzer, 1992a; Whittle, 1993). We previously showed that indomethacin, injected at a dose preventing the stimulation of gastric PGE_2 formation elicited by ic injection of TRH-analog (Yanagisawa 1990b), did not influence the increase in GMBF induced by RX 77368 either at 1.5 or 30 ng (Kiraly 1997b; Tanaka 1997). Likewise, electrical vagal stimulation- or intra-

arterial injection of acetylcholine-induced gastric hyperemia is not altered by indomethacin in rats (Kitagawa 1987a; Kiraly 1997b). The present study shows that pyrilamine, infused at a dose which blocked H_1-mediated vascular changes (Holm 1994), did not inhibit the GMBF response to ic injection of RX 77368. The H_2 agonist, impromidine, does alter GMBF in rats at doses that stimulates acid secretion and intra-arterial infusion of histamine does not influence gastric vascular resistance (Holm 1994). Therefore, these data indicate that gastric histamine released by ic RX 77368 at 30 ng (Yanagisawa 1990a) is unlikely to play a role in gastric vascular changes induced by RX 77368. In addition, capsaicin, which is known to deplete CGRP from gastric afferent fibers (Holzer, 1992a), did not prevent the gastric hyperemic response to ic injection of TRH analog at a 30 ng dose (Kiraly 1997b), present observation. By contrast, capsaicin and intravenous injection of the CGRP antagonist prevented the increase in GMBF elicited by ic injection of RX 77368 at a lower dose (1.5 ng) (Kiraly 1997b). Therefore, these findings indicate that additional mechanisms come into play at a higher intensity of central vagal activation which increases GMBF.

The present findings support a primary role of peripheral L-arginine-NO synthase pathways in the muscarinic dependent stimulation of GMBF and decreases vascular resistance evoked by ic RX 77368 at 30 ng.

First, the organization of the gastric circulation requires submucosal arterioles to be dilated to increase GMBF (Guth 1987) and NO exerts an important vasodilatory influence in the gastric circulation (Pique 1989; Chen 1993).

Second, RX 77368 injected ic at 30 ng stimulates gastric NO generation through vagal atropine-sensitive pathways (Saperas 1995).

Lastly, the NO synthase inhibitor, L-NAME injected iv completely abolished gastric hyperemia in response to ic

injection of RX 77368 at 30 ng dose in intact (Tanaka 1993) and capsaicin-pretreated rats (present study). L-NAME was reported to compete non-selectively at the agonist binding site of several forms of muscarinic receptors in *in vitro* assays (Buxton 1993). However, L-NAME action results from peripheral inhibition of NO synthase activity rather than a possible anti-muscarinic action. L-NAME inhibitory action in capsaicin-pretreated rats was reversed in a specific manner by an excess of L-arginine, a substrate for NO synthase while the D-arginine enantiomer was inactive. Moreover, L-NMMA, an inhibitor of endothelial NO synthase, which is devoid of muscarinic antagonist properties (Rees 1990; Buxton 1993), also blocked the increase in GMBF and decrease in GMVR induced by RX 77368 in intact rats. Lastly, L-NAME injected into the cisterna magna did not alter the gastric vascular responses to ic TRH analog. The biological activity of L-NAME injected ic was supported by the increase in basal MAP as previously observed using ic injection of other NO synthase inhibitors (Togashi 1992). Therefore, although systemic injection of L-NAME has been reported to decrease NO synthase in the rat forebrain (iadecola 1994), a peripheral action is responsible for L-NAME-induced blockade of the gastric vascular responses to ic RX 77368.

The source of enhanced NO production most likely originates from endothelium where NO is abundantly synthesized (Palmer 1988). In addition, acetylcholine increases GMBF through endothelium-dependent mechanisms (Kitagawa 1987a) and direct superfusion of low doses of acetylcholine onto submucosal arterioles induced vasodilation which is prevented by L-arginine-NO pathways blockade (Chen 1993). Nitric oxide synthase is also found in postganglionic neurons of the parasympathetic system that innervate the stomach (Forster 1993a). The neuronal NO serves as a peripheral non-cholinergic and non-adrenergic transmitter of gastric relaxation induced by high frequency electrical vagal stimulation (Lefebvre 1992). However, since atropine completely abolished the NO-dependent

increase in GMBF induced by ic RX 77368, it is unlikely that neuronal NO contributes to the gastric vascular response.

Previous reports consistently showed that the hypertensive response to intracerebroventricular TRH is mediated by the sympathetic nervous system and catecholamine release while parasympathetic cholinergic mechanisms do not play a role (Mattila 1986; Siren 1988; Thiefin 1989). Likewise, atropine did not alter the rise in MAP induced by ic injection of TRH analog at 30 ng. We also found that the increase in MAP induced by ic RX 77368 was not influenced by an excess of L-arginine. These results indicate that the hypertensive response to ic TRH analog is not related to autonomic mediated alterations of tonic NO generation in peripheral vascular beds (Lacolley 1991). By contrast, the enhanced blood pressure response to ic RX 77368 by systemic injection of NO inhibitors was reversed in an enantiomeric manner by an excess of L- but not D-arginine. These results most likely reflect the additional increase in peripheral vascular resistance in various beds due to the removal of the continuous vasodilatory action of NO when NO synthase inhibitors are injected into the circulation (Pique 1989; Rees 1989). Atropine, L-NAME or L-NMMA blocked the decrease in gastric vascular resistance induced by ic RX 77368 while the increase in MAP was unchanged by atropine and further enhanced by NO inhibitors. These results further indicate that the increase in GMBF induced by central RX 77368 is not merely the result of changes in systemic arterial pressure but is likely to represent muscarinic-NO mediated vasodilatation occurring locally in the gastric submucosal arterioles.

In summary, the present observations demonstrate that ic injection of RX 77368 at 30 ng increases GMBF through atropine-sensitive mechanisms. In addition, NO, most likely originating from vascular endothelial cells, plays a crucial role in mediating the muscarinic-dependent stimulation of GMBF induced by central vagal activation in intact or capsaicinized rats. By contrast, other vasodilatory agents, namely histamine acting on

H_1 receptor or indomethacin (Tanaka 1997) are not involved. Since medullary TRH plays a physiological role in the vagal regulation of gastric function (Tache 1994a), these data may have a bearing on the underlying mechanisms through which GMBF is increased in response to central vagal activation. These studies also point out that central vagal stimulation by TRH may be a more relevant tool to assess the vagal regulation of gastric circulation compared with electrical vagal stimulation.

3.2.2.5 Perspectives

The present data demonstrate that central vagal efferent activation stimulates GMBF and decreases gastric vascular resistance through cholinergic and NO dependent pathways in rats. Further studies are needed to delineate the precise mechanisms by which central vagal efferent cholinergic activation is coupled or gated to the L-arginine-NO pathways in the gastric submucosal arterioles. Direct superfusion of low concentrations of acetylcholine on gastric submucosal arterioles dilate the arterioles through atropine sensitive and L-arginine-NO dependent mechanisms (Morishita 1986; Chen 1993). Therefore, acetylcholine released from post ganglionic neurons by central vagal activation may diffuse to the endothelium of gastric submucosal arterioles. As recent studies suggest differential coupling of M_1, M_3 and M_5 muscarinic receptors to activate nitric oxide synthase (Wang 1996), muscarinic receptor subtypes which are involved in the generation of NO leading to the dilation of arterioles in the gastric mucosa and increased blood flow will need to be characterized.

3.2.3 Mechanisms mediating gastric hyperemic and acid responses to central TRH analog at a cytoprotective dose

3.2.3.1 Background

TRH or the stable TRH analog, RX 77368, injected intracisternally or into the dorsal vagal complex results in a vagal cholinergic modulation of the resistance of the mucosa to gastric injury (Yoneda 1992; Yoneda 1993b; Tache 1994b; Kaneko 1995a; Kato 1996). In particular, recent studies indicate that RX 77368 or TRH protects the gastric mucosa against ethanol lesions when injected intracisternally or into the dorsal motor nucleus of the vagus at low, but not high doses (Yoneda 1992; Kaneko 1995a; Kaneko 1995b; Tanaka 1997).

Gastric mucosal microcirculation is an important factor in the resistance of the gastric mucosa against injury, particularly as it relates to ethanol-induced gastric lesions (Guth 1987; Whittle, 1993). The maximal effective intracisternal dose of RX 77368 (1.5 ng) at which gastric protection against ethanol injury occurs is subthreshold to increase gastric acid secretion and intraluminal pressure although it stimulates vagal efferent activity (Yoneda 1992; Raybould 1990; O-Lee 1997; Yoneda 1993b). Vagal activation is known to increase GMBF (Guth 1987), however, whether GMBF is enhanced by RX 77368 at a cytoprotective dose is not known. The underlying mechanisms preventing acid secretion in the presence of vagal stimulation also compel further investigations.

We previously showed that the mechanisms involved in the gastric protection against ethanol lesions induced by RX 77368 at 1.5 ng is atropine sensitive and prostaglandin (PG), calcitonin gene-related peptide (CGRP) and nitric oxide dependent (Yoneda 1993b; Yoneda 1992; Kato 1994; Kiraly 1993). There is evidence that CGRP, the predominant neuropeptide localized in primary capsaicin sensitive afferent neurons in the rat stomach (Sternini 1987) and PGE_2 have vasodilatory properties in the gastric mucosal

microcirculation (Guth 1987; Whittle, 1993) and inhibit gastric acid secretion (Yoneda 1993b). Therefore, the aims of the present study were: (1) to examine the influence of intracisternal injection of a low dose of TRH analog (1.5 ng) on GMBF, gastric mucosal vascular resistance (GMVR) and gastric acid secretion monitored simultaneously in urethane-anesthetized rats. (2) to assess the role of prostaglandins and CGRP contained in capsaicin sensitive afferent fibers (Sternini 1987) in mediating or modulating GMBF and gastric secretory responses to intracisternal injection of RX 77368 at 1.5 ng.

3.2.3.2 Materials and methods

Drugs and Treatments

The following substances were used: the stable TRH analog, RX 77368, p-Glu-His-(3,3'-dimethyl)-Pro-NH_2 (Ferring Pharmaceuticals Ltd., Feltham, Middx, U.K.), indomethacin, (Sigma Chemical Co., St. Louis, MO), capsaicin (Sigma), and $hCGRP_{8-37}$ (kindly donated by Dr. S. St. Pierre, Department of Biochemistry, University of Québec, Montréal, PQ, Canada). RX 77368 was aliquoted in 0.1% bovine serum albumin (BSA) and 0.9% saline at a concentration of 3 µg/10 µl, and kept frozen at -70 °C. The stock solution of RX 77368 was dissolved in 0.9% saline (pH 7.0) before intracisternal injection delivered in 10 µl followed by 10 µl of saline to flush the catheter. Indomethacin was dissolved in 1% sodium-bicarbonate and $hCGRP_{8-37}$ in 0.1% BSA containing 0.9% saline. Capsaicin was dissolved in vehicle (absolute ethanol, Tween 80 and 0.9% saline, 10:10:80:v/v/v).

Animal Preparations and Surgical Procedures

All the surgical procedures performed for simultaneous measurements of GMBF, MAP, and gastric acid secretion and drug administration were essentially the same as previously described in chapter 3.2.

Simultaneous Measurements of GMBF, MAP, and Acid Secretion

After an equilibration period of 1 h, GMBF was measured by the hydrogen gas-clearance technique as previously described in chapter 3.2. In the same preparation, the gastric effluent was continuously collected and separated into 15 min fractions which were titrated with 0.01 N NaOH using an automatic titrator (Radiometer Copenhagen). Gastric acid output was expressed in μmol/30 min.

Experimental Protocols

In all the experiments, repeated monitoring of GMBF was performed twice under basal conditions, and twice again for the 30 min periods before and after intracisternal injection of RX 77368 (1.5 ng). Gastric acid output reflects the 30 min periods before pretreatment (basal) and before and after intracisternal injection of RX 77368. MAP values were selected at 15 min before and after intracisternal injection RX 77368 as used for the calculation of GMVR.

Study 1. Effect of indomethacin.

After a 60 min basal period, indomethacin (5 mg/kg) or vehicle (1% $NaHCO_3$) was injected intraperitoneally in 0.3 ml and 60 min later, RX 77368 (1.5 ng) was injected intracisternally. The dose of indomethacin was selected based on the inhibition of gastric PGE_2 release induced by intracisternal injection of RX 77368 at 1.5 ng in urethan-anesthetized rats (Yoneda 1992; Yoneda 1993b). Parameters were monitored during the basal period, and 30 min periods before and after administration of RX 77368.

Study 2. Effect of capsaicin.

Rats were injected subcutaneously 3 times at 12-h intervals with either capsaicin (25, 50 and 50 mg/kg, 0.5 ml) or vehicle (absolute ethanol, Tween 80 and isotonic saline, 10:10:80:v/v/v, 0.5 ml). The first capsaicin injection was performed under short

enflurane anesthesia. After 10-14 days, all the capsaicin-pretreated rats showed the disappearance of the corneal chemosensory reflex (eye wiping for 1-3 min) to a drop of 0.1% NH_4OH instillated into each eye when tested just before the experiment. After a 60 min basal period, RX 77368 (1.5 ng) was injected intracisternally in vehicle- and capsaicin-pretreated rats. Parameters were monitored for 30 min periods before and after RX 77368 administration.

Study 3. Effect of CGRP antagonist.

After a 60 min basal period, vehicle (0.1% BSA/saline: 0.3 ml followed by 1.5 ml/h) or $hCGRP_{8-37}$ (15 μg/kg bolus followed by 3 μg/kg/h) was infused intravenously throughout the experimental period. The intracisternal injection of RX 77368 (1.5 ng) was performed 60 min after the start of the iv infusion of vehicle or CGRP antagonist. The regimen of $hCGRP_{8-37}$ administration was based on our previous study showing the complete inhibition of intra-arterial α-CGRP-induced gastric mucosal hyperemia in urethan-anesthetized rats (Kiraly 1994). Parameters were measured during the basal period and the 30 min periods before and after RX 77368 administration.

Statistics

Results are expressed as means ± SEM. Comparisons between before and after intracisternal injection of RX 77368 were analyzed by Student's paired t-test. Comparisons between two groups were performed by Student's t-test. Multiple group comparisons were performed by ANOVA followed by Duncan's contrast. $P<0.05$ was considered statistically significant.

3.2.3.3 Results

Study 1. Effect of Indomethacin on the GMBF, GMVR, MAP and Acid Responses to RX 77368

In vehicle-pretreated groups, intracisternal injection of the TRH analog, RX 77368 at 1.5 ng resulted in a significant increase in GMBF values (ml/min/100 g) from 46.8 ± 5.3 to 100.6 ± 20.9 during the periods 15 min before and 15-30 min after intracisternal RX 77358 injection respectively (Fig. 11). During the 45-60 min post injection, GMBF was returning to basal levels (67.7 ± 8.5 ml/min/100 g, n=6). MAP values (mm Hg) were 70.3 ± 2.1 at 15 min before intracisternal injection and rose to 84.3 ± 5.9 at 15 min after RX 77368 injection (Fig. 12). The onset of hypertensive response was rapid (within 1-2 min) and plateaued thereafter to return to basal levels 30-45 min after TRH analog injection (data not shown). GMVR (mmHg/ml/min/100 g) decreased from 1.50 ± 0.33 to 0.84 ± 0.08 during the periods 15 before and 15-30 min after intracisternal injection of RX 77368 respectively (Fig. 12). Gastric acid secretion (μmol/30 min) did not change significantly before (1.8 ± 0.4) and after (4.7 ± 1.7) intracisternal RX 77368 injection (Fig. 11). As previously reported (Kiraly 1994), intracisternal injection of saline in vehicle-pretreated rats did not change GMBF (ml/min/100 g: 51.7 ± 4.7 and 50.7 ± 5.5 during the periods 15 min before and 15-30 min after saline respectively), GMVR (mmHg/ml/min/100 g: 1.83 ± 0.13 and 1.85 ± 0.14 during the periods 15 min before and 15-30 min after saline respectively) or MAP (mmHg: 94 ± 2 and 93 ± 3 at 15 min before and after saline).

Compared with vehicle pretreatment, indomethacin (5 mg/kg, ip) did not influence basal GMBF, MAP and GMVR and tends to reduce basal gastric acid secretion in urethan-anesthetized rats (Figs. 11, 12). Indomethacin did not modify intracisternal RX 77368 (1.5 ng)-induced gastric hyperemia (Fig. 11), rise in MAP and decrease in GMVR during the 15-30 min period post injection

(Fig. 12). However, intracisternal injection of RX 77368 (1.5 ng) induces a robust increase in gastric acid secretion in indomethacin-pretreated rats (19.7 ± 2.9 μmol/30 min) while there was no significant increase (4.7 ± 1.7 μmol/30 min) after RX 77368 in vehicle-pretreated group (Fig. 12).

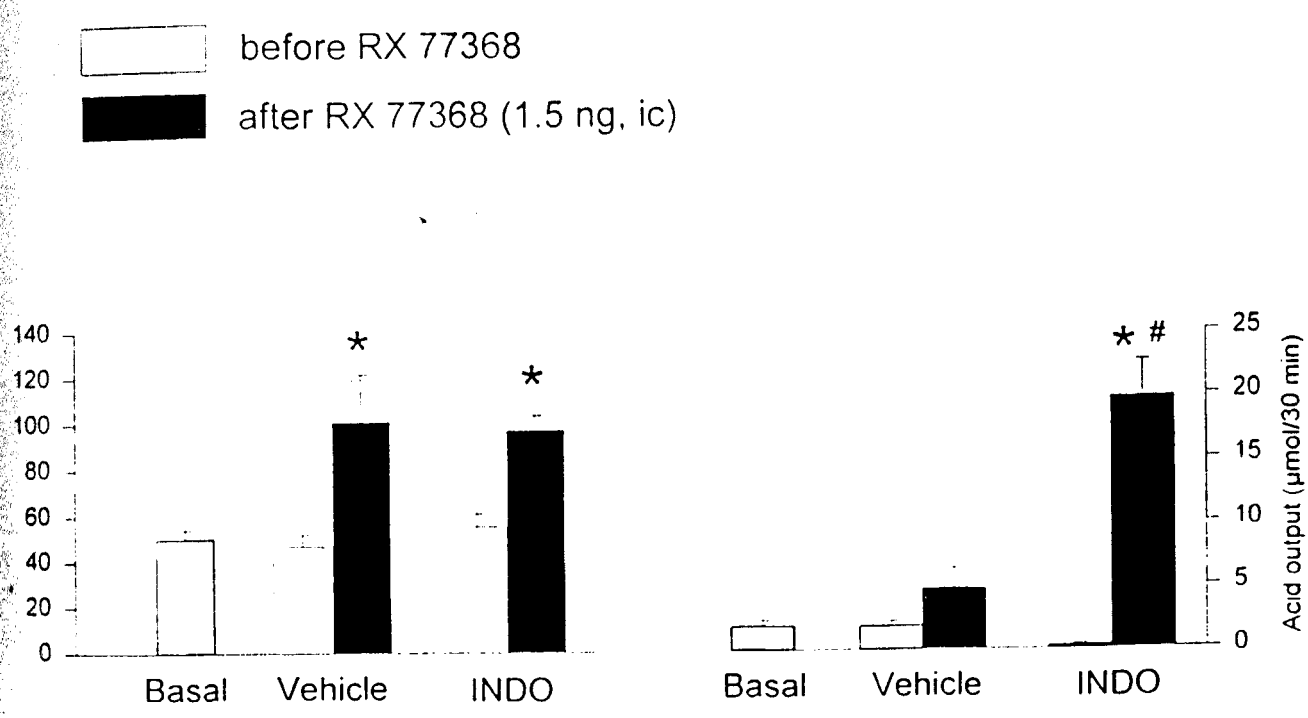

Fig. 11. Effect of indomethacin (IND) on gastric mucosal blood flow (GMBF) and acid responses to 1.5 ng dose of RX 77368 injected intracisternally (ic). IND (5 mg/kg, ip) or vehicle (0.3 ml, sc) was injected 60 min before RX 77368 (1.5 ng, ic). Each column represents the mean ± SEM of 4-10 rats/group. * $P < 0.05$ (paired t-test) compared with respective values before RX 77368 injection; # $P < 0.05$ (unpaired t-test) compared with vehicle plus RX 77368-treated group. GMBF values are during the 15 min period before IND or vehicle (basal), 15 min period before intracisternal injection (before RX 77368) and 15-30 min after intracisternal injection (after RX 77368); acid secretion values are 30 min period before IND or vehicle (basal) and before and after RX 77368.

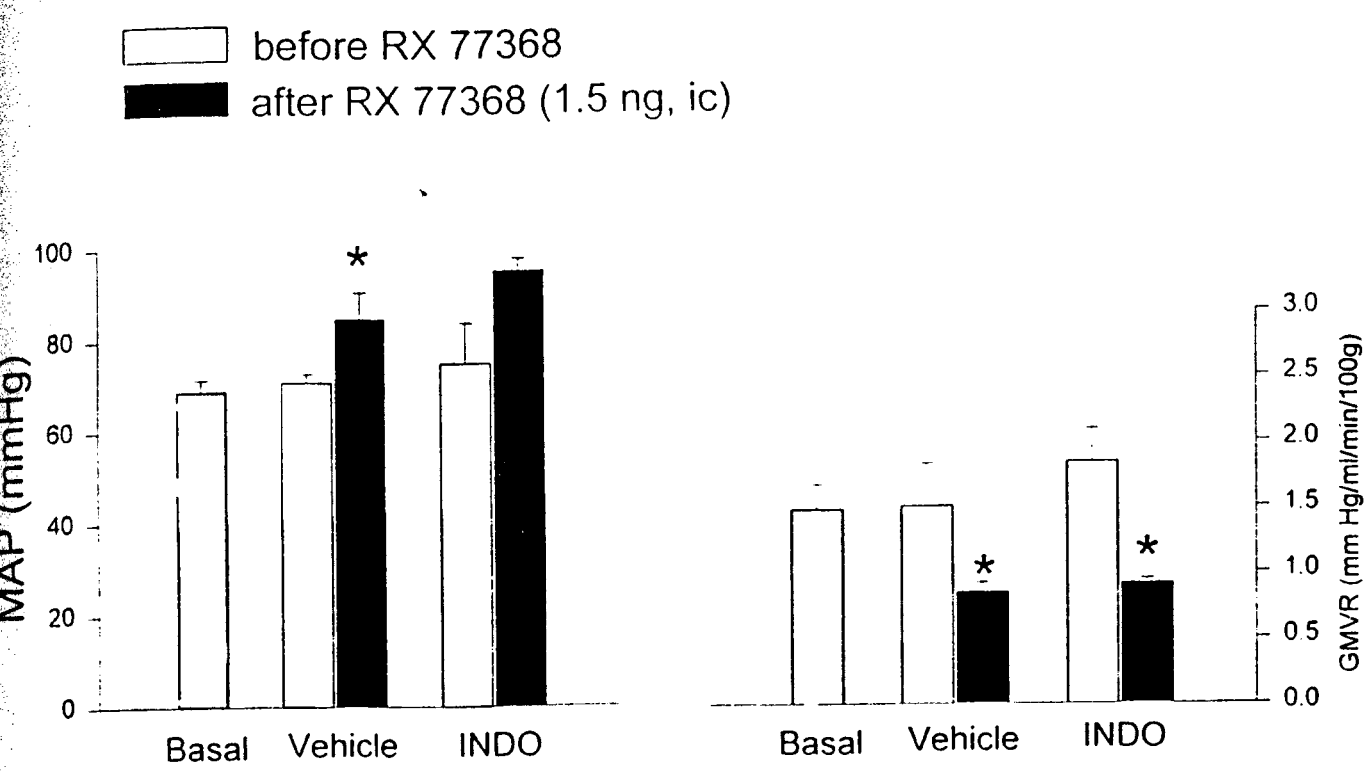

Fig. 12. Effect of indomethacin (IND) on mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) responses to 1.5 ng of RX 77368 injected intracisternally (ic). Similar treatments and animals as described in Fig. 11. Each column represents the mean ± SEM. * P< 0.05 (paired t-test) compared with respective values 30 min before RX 77368 injection. MAP values are at 15 min before IND or vehicle (basal) and at 15 min before or after RX 77368; GMVR values are during the 15 min period before IND or vehicle (basal), 15 min period before intracisternal injection before and 15-30 min after intracisternal injection of RX 77368.

Study 2. Effect of capsaicin on the GMBF, GMVR, MAP and acid responses to RX 77368

Systemic capsaicin pretreatment did not alter the basal GMBF, MAP, GMVR and gastric acid secretion compared with vehicle-pretreated rats (Figs. 13, 14). The gastric hyperemia observed during the 15-30 min period after intracisternal injection of RX 77368 (1.5 ng) was abolished by capsaicin-pretreatment (Fig. 13) while the rise in MAP was not modified (Fig. 14). Intracisternal RX 77368 which decreases GMVR in vehicle-pretreated rats, induced a significant elevation in GMVR in capsaicin-pretreated rats (Fig. 14). During the 30 min period after intracisternal RX 77368 (1.5 ng), there was also a significant increase in gastric acid output (8.7 ± 2.7 μmol/30 min) in capsaicin-pretreated rats compared with preinjection levels (1.6 ± 0.7 μmol/30 min) (Fig. 13). In the vehicle-pretreated group, the 30 min gastric acid outputs before and after intracisternal injection of RX 77368 were not significantly different (from 2.9 ± 0.8 to 6.2 ± 1.7 μmol/30 min) (Fig. 13).

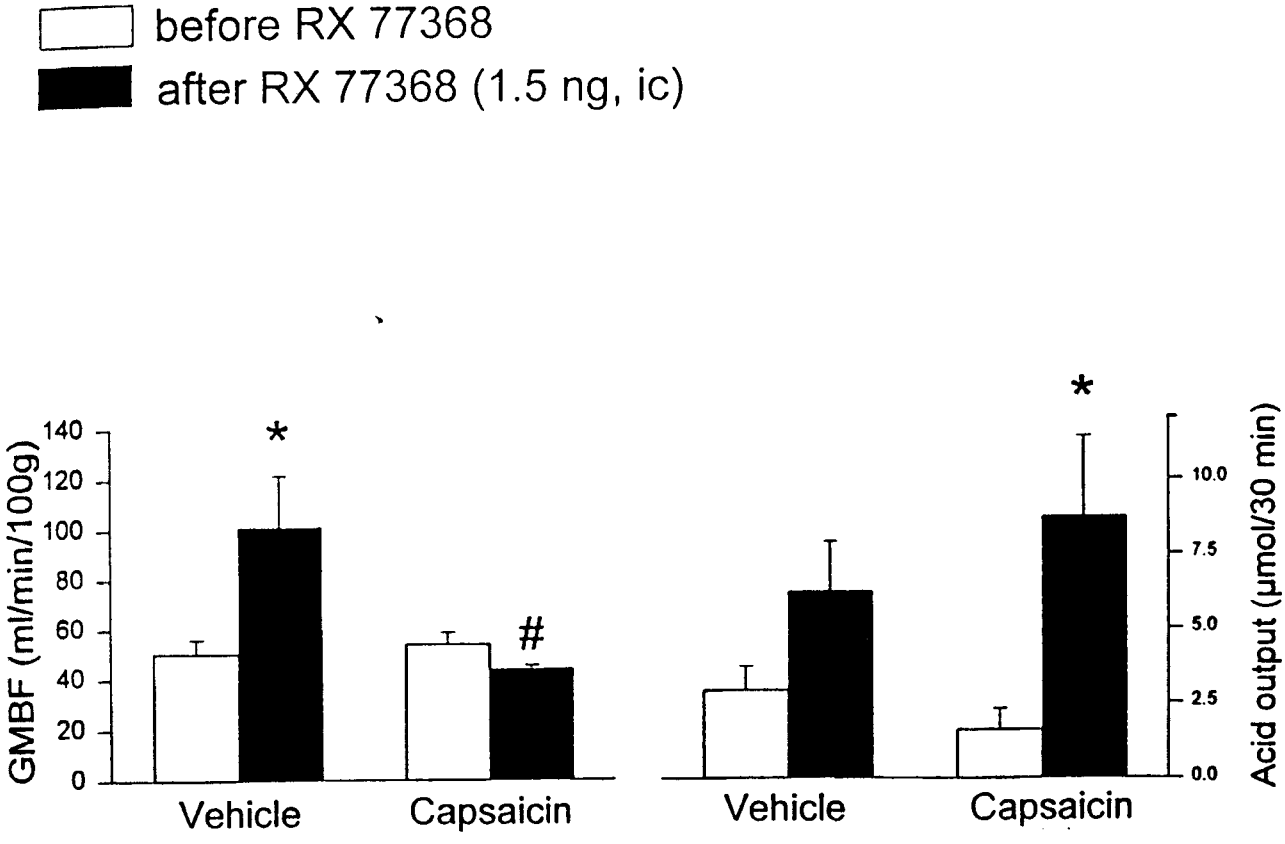

Fig. 13. Effect of sensory nerve ablation by systemic capsaicin pretreatment on gastric mucosal blood flow (GMBF), and acid responses to 1.5 ng of RX 77368 injected intracisternally (ic). Capsaicin or vehicle was given s.c. at doses of 25, 50, 50 mg/kg on three consecutive days 10-14 days before RX 77368 administration. Each column represents mean ± SEM of 4-6 rats/group. * $P< 0.05$ (paired t-test) compared with respective basal values; # $P< 0.05$ (unpaired t-test) compared with vehicle plus RX 77368-treated group. GMBF values are during the 15 min period before and 15-30 min after intracisternal injection of RX 77368; acid secretion values are 30 min period before and after RX 77368.

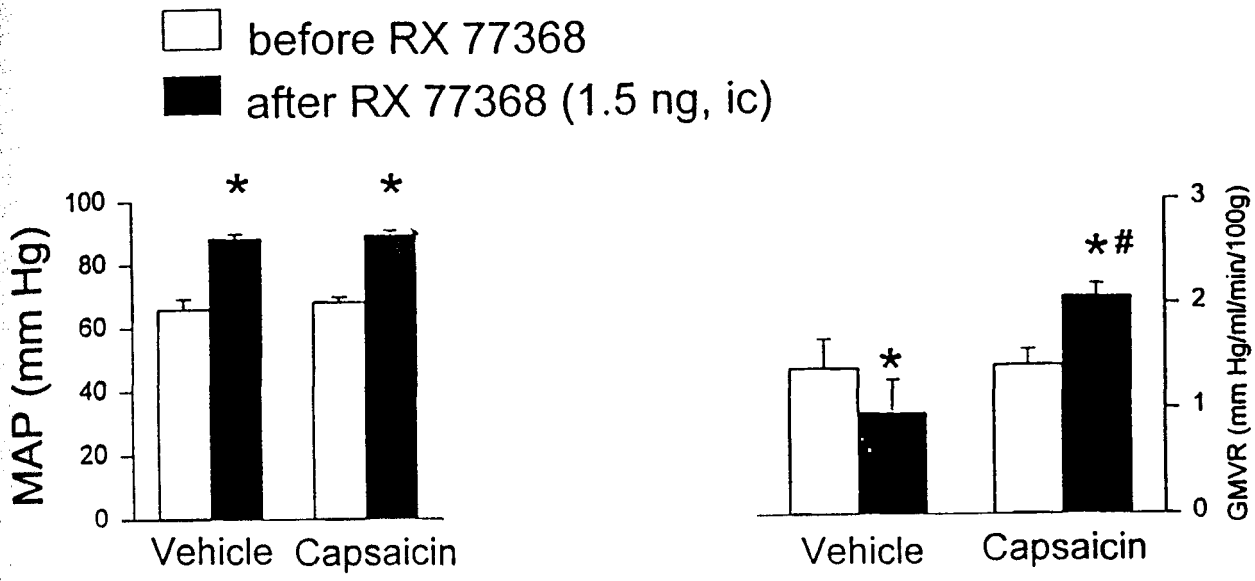

Fig. 14. Effect of sensory nerve ablation by systemic capsaicin pretreatment on mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) responses to 1.5 ng of RX 77368 injected intracisternally (ic). Similar treatment and animals as described in Fig. 14. Each column represents mean ± SEM of 4-6 rats/group. * $P < 0.05$ (paired t-test) compared with respective basal values; # $P < 0.05$ (unpaired t-test) compared with vehicle plus RX 77368-treated group. MAP values are at 15 min before or after RX 77368; GMVR values are during the 15 min period before intracisternal injection (before RX 77368) and 15-30 min after intracisternal injection (after RX 77368).

Study 3. Effect of CGRP receptor antagonist, $hCGRP_{8-37}$ on the GMBF, GMVR, MAP and acid responses to RX 77368

The CGRP receptor antagonist, $hCGRP_{8-37}$ (15 μg/kg iv bolus then infused intravenously (3 μg/kg/h) for 60 min before and after intracisternal injection of TRH analog), did not influence basal values of GMBF, MAP, GMVR and gastric acid secretion compared with vehicle-pretreated group (Figs. 15, 16). However, $hCGRP_{8-37}$ infused intravenously prevented RX 77368-induced stimulation of GMBF (Fig. 15) and the decrease in GMVR (Fig. 16) while the rise in MAP was maintained (Fig. 16). In the presence of CGRP receptor antagonist, intracisternal TRH analog tends to increase gastric acid secretion (27.3 ± 14.1 μmol/30 min) compared with pre-injection values (6.6 ± 3.5 μmol/30 min) and GMVR (mmHg/ml/min/100g: from 1.60 ± 0.17 to 2.01 ± 0.25) although the differences did not reach statistical significance (Figs. 15, 16).

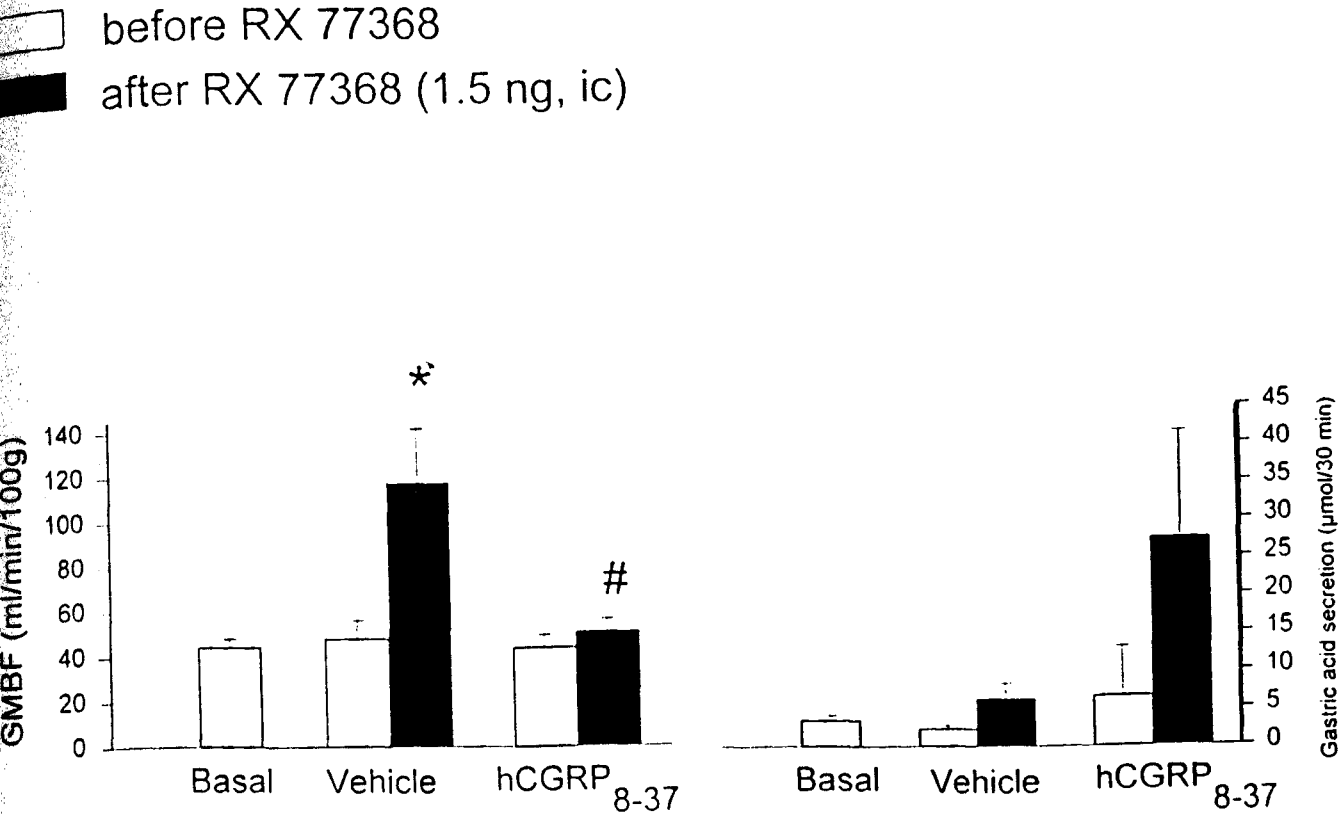

Fig. 15. Effect of $hCGRP_{8-37}$ on gastric mucosal blood flow (GMBF) and acid responses to 1.5 ng of RX 77368 injected intracisternally (ic). $CGRP_{8-37}$ (15 µg/kg + 3 µg/kg/h iv) or vehicle (0.3 ml + 1.5 ml/h, iv) was started to inject 60 min before RX 77368 administration. Each column represents the mean ± SEM of 4-6 rats/group. * $P<0.05$ (paired t-test) compared with respective basal values; # $P< 0.05$ (unpaired t-test) compared with vehicle plus RX 77368-treated group. GMBF values are during the 15 min period before $hCGRP_{8-37}$ or vehicle (basal), 15 min period before intracisternal injection (before RX 77368) and 15-30 min after intracisternal injection (after RX 77368); acid secretion values are 30 min period before $hCGRP_{8-37}$ or vehicle (basal) and before and after RX 77368.

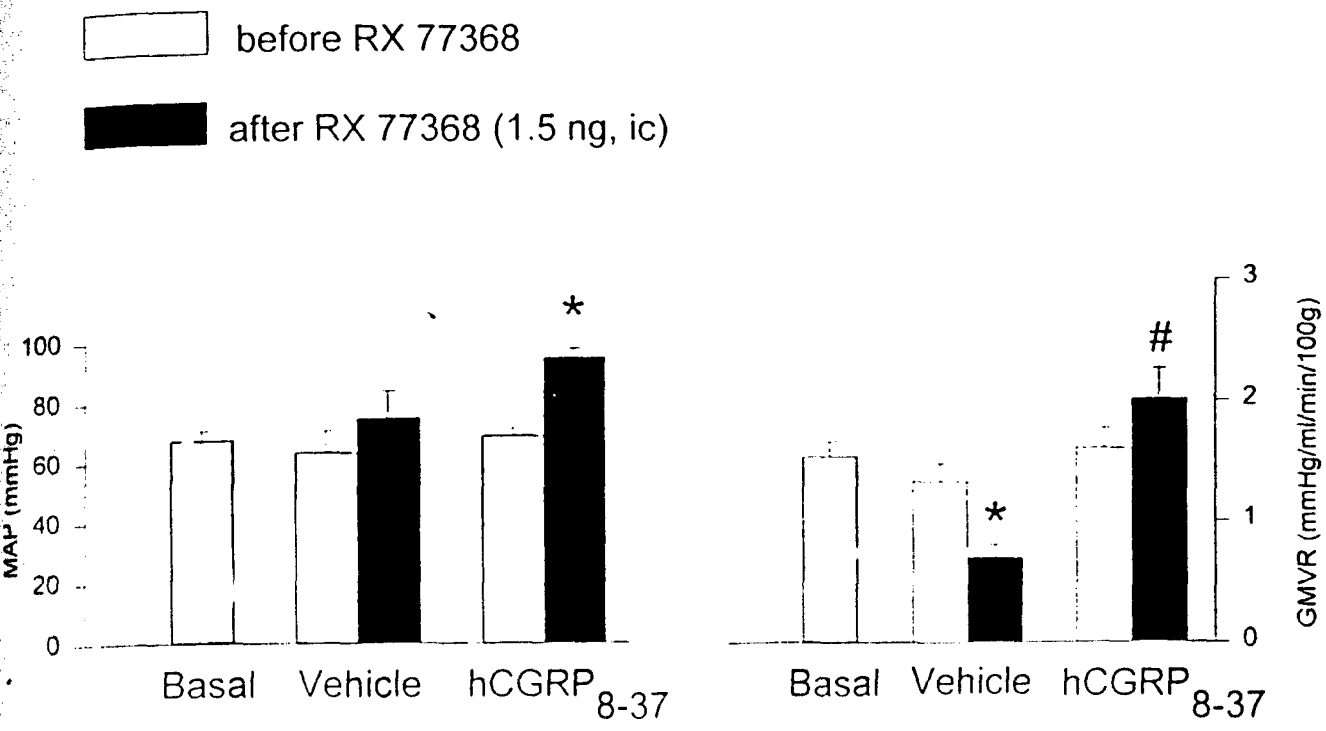

Fig. 16. Effect of $hCGRP_{8-37}$ on mean arterial pressure (MAP) and gastric mucosal vascular resistance (GMVR) to 1.5 ng of RX 77368 injected intracisternally (ic). Similar treatments and animals as described in Fig. 15. Each column represents the mean ± SEM of 4-6 rats/group. * $P<0.05$ (paired t-test) compared with respective basal values; # $P< 0.05$ (unpaired t-test) compared with vehicle plus RX 77368-treated group. MAP values are at 15 min before $hCGRP_{8-37}$ or vehicle (basal) and at 15 min before or after RX 77368; GMVR values are during the 15 min period before $hCGRP_{8-37}$ or vehicle (basal), 15 min period before intracisternal injection (before RX 77368) and 15-30 min after intracisternal injection (after RX 77368).

3.2.3.4 Discussion

The TRH analog, RX 77368, injected intracisternally at 1.5 ng stimulated GMBF in urethan-anesthetized rats as measured within the 15-30 min period post injection by the hydrogen gas clearance technique. Peptide action at 1.5 ng was shorter lasting (less than 60 min) compared with the 30 ng dose tested under the same conditions (Tanaka 1993; Kiraly 1994). The GMBF response represents a specific effect of the peptide as intracisternal injection of vehicle did not modify GMBF as reported previously (Kiraly 1994). Convergent evidence in urethan-anesthetized rats indicates that the gastric hyperemia induced by the central injection of TRH or RX 77368 is mediated by vagal cholinergic dependent mechanisms (Thiefin 1989; Kiraly 1994; Kiraly 1998).

First, RX 77368 injected intracisternally at 1.5 ng stimulates gastric vagal efferent discharge within 5 min for over 30 min post injection (Thiefin 1989; Tanaka 1993; Kiraly 1994; Kiraly 1998).

Second, the gastric hyperemia can be evoked by the direct microinjection of TRH into the dorsal motor nucleus of the vagus (O-Lee 1997) and abolished by vagotomy and peripheral, but not central, injection of atropine (Fantozzi 1978; Thiefin 1989). In addition, intravenous administration of the cholinergic agonist, bethanechol, mimics the increase in GMBF elicited by central vagal stimulation (Kiraly 1994). This contrasts with the gastric hyperemic response to electrical stimulation of the vagus which, in addition to cholinergic dependent mechanisms, involves an atropine-resistant component (Guth 1987).

The peripheral mechanisms by which intracisternal RX 77368 at a low dose increases GMBF are not secondary to changes in acid secretion and prostaglandin release. In previous studies, TRH or RX 77368 was injected intracisternaly at doses which stimulated both gastric acid secretion and GMBF (Thiefin 1989; Tanaka 1993; Kiraly 1994). There are examples of changes in regional GMBF in response to stimulation of gastric acid secretion (Guth 1987).

However, the stimulation in GMBF induced by RX 77368 at 1.5 ng occurs in the absence of a significant stimulation of gastric acid secretion as monitored simultaneously in the same animal preparation. RX 77368 injected intracisternally or into the dorsal motor nucleus of the vagus at low doses stimulates the gastric release of PGE_2 which is blocked by vagotomy (Yoneda 1993b; Kato 1995b). PGE_2 is known to have a vasodilatory effect on the gastric mucosal microvessels (Guth 1987; Whittle, 1993). However, indomethacin injected at a dose which suppresses the stimulation of gastric PGE_2 release in response to intracisternal RX 77368 at 1.5 ng (Yoneda 1993b), did not modify its gastric hyperemic response. Likewise, indomethacin did not influence the increase in GMBF (measured by the oxygen saturation of hemoglobin) evoked by electrical stimulation of the cervical vagus or intra-arterial injection of acetylcholine in rats (Kitagawa 1987b).

Convergent functional and anatomical evidence indicates that the CGRP contained in capsaicin sensitive afferent fibers plays an important role in the gastric hyperemia to RX 77368 injected intracisternally at 1.5 ng.

First, CGRP exerts a potent local vasodilatory effect in the gastric submucosal arterioles in rats (Chen 1992).

Second, gastric submucosal arterioles are densely innervated by capsaicin sensitive sensory nerves containing CGRP (Sternini 1987).

Lastly, both, capsaicin pretreatment or intravenous injection of $hCGRP_{8-37}$ completely prevented the 100% increase of GMBF and decrease in gastric vascular resistance induced by intracisternal RX 77368 at 1.5 ng. Systemic capsaicin pretreatment was shown to ablate CGRP immunoreactivity contained in splanchnic neurons innervating gastric blood vessels (Sternini 1987). $hCGRP_{8-37}$ is a specific CGRP receptor antagonist (Dennis 1990) previously reported to abolish intravenous CGRP-induced gastric hyperemia in rats (Kiraly 1994). The lack of effect of

these treatments on basal GMBF, as previously reported (Kiraly 1994), indicate that CGRP contained in capsaicin sensitive afferents does not contribute to the basal regulation of GMBF in urethan-anesthetized rats. Close intra-arterial infusion of the muscarinic agonist, bethanechol, increases GMBF and the gastric hyperemia was abolished by intravenous infusion of the CGRP antagonist in urethan-anesthetized rats (Kiraly 1994). Likewise in the perfused mesenteric vascular beds isolated from rats, acetylcholine induced a prolonged vasodilation mediated by CGRP release from capsaicin sensitive afferents (Takenaga 1995). These data along with the demonstration that gastric circulation requires submucosal arterioles to be dilated to increase GMBF (Guth 1987), suggest that a cholinergic-CGRP dependent vasodilation of gastric arterioles may play an important role in the gastric hyperemic response to central RX 77368 at 1.5 ng (Fig. 17.).

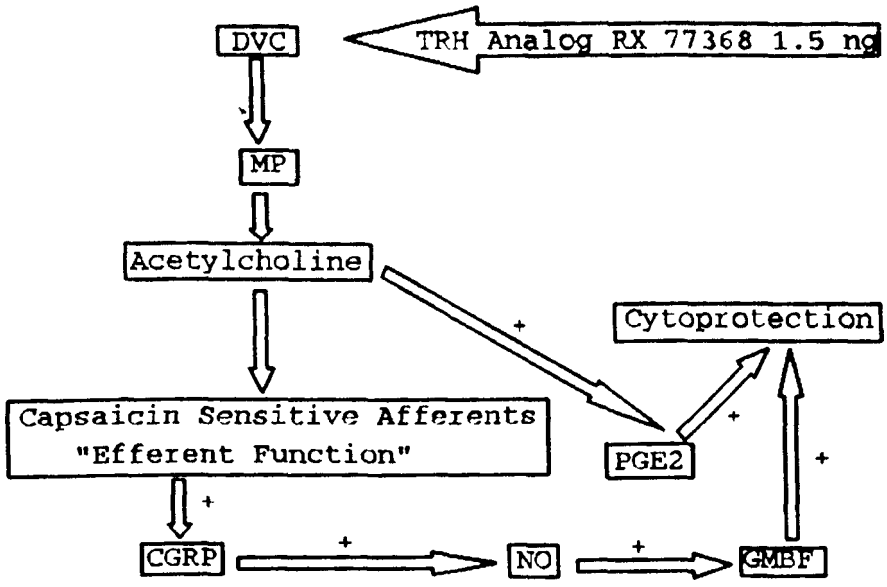

Fig. 17. Schematic representation of the action of RX 77368 injected at a cytoprotective dose (1.5 ng ic) in the dorsal vagal complex (DVC) to induce vagal muscarinic dependent stimulation of gastric secretion of histamine, prostaglandin F_2 (PGF_2) calcitonin gene-related peptide (CGRP) nitric oxide (NO) and gastric mucosal blood flow (GMBF)

We recently reported that intracisternal injection of RX 77368 at a maximal gastric secretory dose (30 ng) stimulated GMBF also through vagal cholinergic and CGRP dependent mechanisms whereas substance P did not play a role (Kiraly 1994). However, in capsaicinized rats, an alternate pathway involving nitric oxide was able to maintain the hyperemic response independently of CGRP (Kiraly 1994) (Fig.18.).

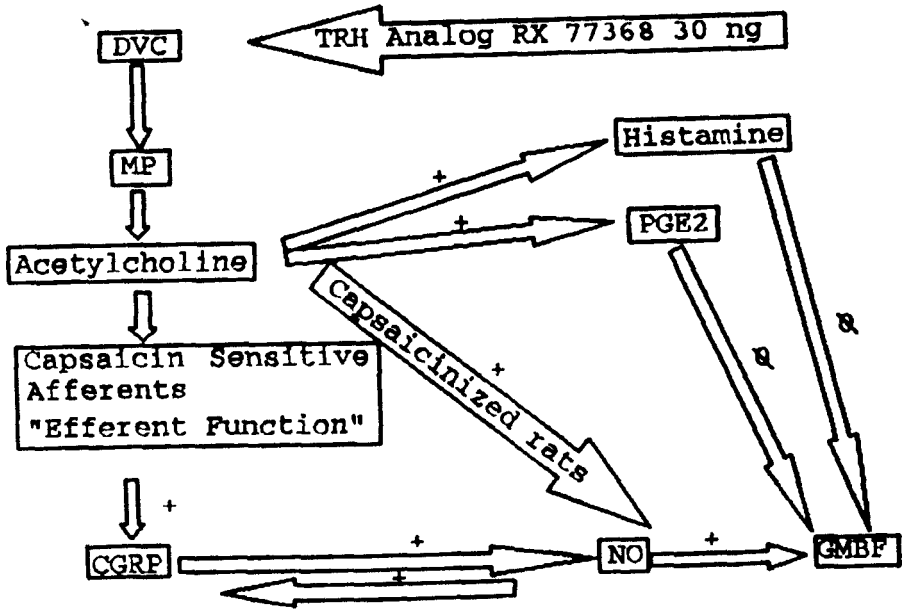

Fig. 18. Schematic representation of the action of RX 77368 injected at a maximal acid secretory dose (30 ng ic) in the dorsal vagal complex (DVC) to induce vagal muscarinic dependent stimulation of gastric secretion of histamine, prostaglandin I_2 (PGI_2) calcitonin gene-related peptide (CGRP) nitric oxide (NO) and gastric mucosal blood flow (GMBF)

By contrast, the present findings show that when RX 77638 is injected intracisternally at a submaximal acid secretory dose, the "efferent function" of capsaicin sensitive afferents releasing CGRP (Holzer, 1988) represents the main underlying mechanism whereby the gastric hyperemic response occurs. Therefore, depending upon the level of central stimulation of the vagus alternate route/or cascades are recruited and come into play (Figs. 17, 18.).

RX 77368 injected intracisternally at 1.5 ng elevated MAP as previously reported for central injection of TRH or RX 77368 at higher doses (Mattila 1986; Thiefin 1989; Kiraly 1994). The hypertensive response is not altered by inhibition of prostaglandins or CGRP contained in capsaicin sensory afferents. Previous pharmacologic and nerve ablation studies indicated that the rise in blood pressure induced by central injection of TRH did not involve vagal cholinergic mechanisms, but is related to changes in sympathetic outflow in rats (Mattila 1986; Siren 1988; Thiefin 1989). In particular, intracerebroventricular injection of TRH increases the splanchnic nerve activity in the adrenal and renal branches as well as elevates circulating levels of catecholamine (Brown, 1981; Somiya 1984; Mattila 1986). By contrast, there is a decrease in splanchnic nerve activity in the gastric branch (Somiya 1984). In capsaicin- or CGRP antagonist-pretreated rats, intracisternal RX 77368 at 1.5 ng increased, instead of decreasing GMVR, suggesting vasoconstriction of gastric vessels. Whether alterations in sympathetic activity and catecholamine release modulate the vagal dependent gastric hyperemic response to intracisternal injection of RX 77368, particularly when the local vasodilatory effect of CGRP is removed, needs to be further investigated.

An adequate level of blood flow is essential for gastric mucosal tissue to withstand the challenge of both endogenous and exogenous aggressors (Whittle, 1993). Intracisternal injection of RX 77358 at 1.5 ng is maximally effective to protect the gastric mucosa against ethanol injury (Kato 1995b). The increase in GMBF

induced by RX 77368 at 1.5 ng may contribute, at least in part, to the gastroprotective effect since capsaicin and CGRP antagonist abolished both the gastric protection against ethanol (Kato 1994; Kato 1995b) and the gastric hyperemia observed at such an intracisternal dose of RX 77368.

While GMBF is increased by intracisternal RX 77368 at 1.5 ng, gastric acid secretion was not significantly modified as previously reported (Raybould 1990; Yoneda 1993b). The lack of an acid secretory response in the presence of an activation of gastric vagal efferent discharges (O-Lee 1997) may result from the inhibitory effect of gastric prostaglandins and CGRP. We previously showed that intravenous injection of PGE_2 or α-CGRP inhibits gastric acid secretion stimulated by vagal cholinergic pathways in urethan anesthetized rats (Saperas 1991; Tache, 1992). Indomethacin induces a robust increase in gastric acid secretion in response to intracisternal RX 77368 at 1.5 ng while there was no significant secretory response to the same dose of RX 77368 in the vehicle-pretreated rats. In addition, depletion of CGRP from gastric afferent fibers by capsaicin pretreatment (Sternini 1987; Raybould 1992) also significantly stimulated gastric acid secretion after RX 77368 while there was no significant increase in vehicle-pretreated rats. Moreover, blockade of CGRP receptors by peripheral infusion of $hCGRP_{8-37}$ tends to increase acid secretion in response to intracisternal RX 77368, although the difference did not reach statistical significance. The dose of CGRP antagonist used (18 μg/kg/h) abolished close intra-arterial infusion of α-CGRP (14 μg/kg/h) and endogenous CGRP-induced increase in GMBF (Kiraly 1994) (present study). However such a dose may be submaximal to prevent endogenous CGRP action on acid secretion. In a previous dose response study, an intravenous dose of 400 μg/kg of $hCGRP_{8-37}$ was required to abolish intravenous injection of α-CGRP (14 μg/kg)- or endogenous CGRP-induced inhibition of gastric acid secretion in urethan-anesthetized rats (Kato 1995a).

Growing evidence indicates that medullary TRH plays a physiological role in the vagal regulation of gastric function and resistance of the gastric mucosa against injury (Kato 1994; Tache 1994a; Kaneko 1995a) suggesting that medullary TRH may be involved in the central vagal regulation of GMBF. The present observations also lend further support to the concept that central vagal cholinergic activation recruits the "local effector function" of capsaicin sensitive afferent fibers containing CGRP in the rat stomach (Holzer, 1988).

In summary, TRH analog injected intracisternally at a low dose protecting the gastric mucosa against ethanol injury and stimulating gastric vagal efferent activity, induced a 100% increase in GMBF for the 30 min period post injection while not stimulating significantly acid secretion. The gastric hyperemic response is mediated by the vasodilatory action of CGRP released from capsaicin sensitive afferent nerves while changes in gastric prostaglandins and acid secretion do not play a role. The lack of acid response is due to the inhibitory influence of vagally released gastric prostaglandins and CGRP. These results indicate a cross talk between vagal efferent activation and capsaicin sensitive splanchnic afferents containing CGRP which has implication in the understanding of peripheral pathways mediating gastric secretory and hyperemic responses to a low level of central vagal activation.

3.2.4 VIP and central TRH-induced vasodilation: influence of a VIP antagonist: [4Cl-D-Phe^{6},Leu^{17}] VIP

3.2.4.1 Background

Vasoactive intestinal peptide (VIP) is well established to induce relaxation of vascular and non vascular smooth muscles (Fahrenkrug, 1993). In particular, Holzer and Guth (Holzer 1991a) showed that close intra-arterial infusion of VIP increases gastric mucosal blood flow (GMBF) at a dose decreasing blood pressure in urethane-anesthetized rats. VIP immunoreactive enteric neurons receive vagal input and project to the gastric muscuralis mucosae, circular smooth muscle and innervate small blood vessels in rats (Berthoud, 1996). High frequency electrical vagal activation stimulates VIP release from the rat stomach through atropine resistant mechanisms (Ito 1988; Ishihara 1992b; Quian 1996). Consistent reports indicate that the gastric vasodilatory response to electrical vagal stimulation involve an atropine-resistant component particularly at high frequencies of vagal activation (Morishita 1986; Kitagawa 1987b; Thiefin 1990). VIP has been reported to be a prime candidate in the vasodilatory response induced by electrical vagal activation of high threshold fibers in various vascular beds as shown by an immunoneutralization strategy with VIP antibody (Bloom 1980; Lundberg 1980).

Medullary thyrotropin releasing hormone (TRH) is involved in the central vagal regulation of gastrointestinal function (Tache 1994a). TRH or the stable TRH analog, RX 77368, injected into the cerebrospinal fluid induces a vagal dependent stimulation of GMBF (Thiefin 1989; Tanaka 1993; Kiraly 1994) and vagal independent increase in arterial pressure (Mattila 1986; Siren 1988; Thiefin 1989; Kiraly 1998). There is pharmacological evidence that VIP is part of the vagal dependent mechanism through which intracerebroventricular injection of TRH stimulates pancreatic exocrine and intestinal secretion (Lenz 1989; Messmer 1993; Lenz

1995). The possible role of VIP in mediating or modulating the hemodynamic changes induced by intracisternal injection of TRH is unknown. Previous studies indicate that the VIP analog, [4Cl-D-Phe^6,Leu^{17}]VIP, is a selective competitive VIP antagonist in several in vitro or in vivo systems (Pandol 1986; Grider 1990; Lenz 1995). Therefore, in the present study, we test first whether [4Cl-D-Phe^6,Leu^{17}]VIP antagonizes VIP-induced systemic and gastric vascular changes in urethane-anesthetized rats. Secondly, we investigated whether the VIP antagonist modulates the gastric hyperemic and hypertensive responses to intracisternal injection of the TRH analog, RX 77368. The TRH analog was used at a dose of 30 ng which evokes a sustained increase in gastric vagal efferent activity, and GMBF in urethane-anesthetized rats (O-Lee 1997).

3.2.4.2 Methods

Drugs

The following substances were used: the stable TRH analog, RX 77368, (p-Glu-His-(3,3'-dimethyl)-Pro-NH_2) (Reckitt & Colman, Kingston-upon-Hill, England), rat VIP (Peninsula, Belmont, CA) and [4Cl-D-Phe^6,Leu^{17}]VIP (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA). The VIP antagonist was synthesized and purified by high performance liquid chromatography. RX 77368 was aliquoted in 0.1% bovine serum albumin (BSA) and 0.9% saline at a concentration of 3 μg/10 μl, and kept frozen at -70 °C. The stock solution was dissolved in 0.9% saline (pH=7.0) before administration. VIP was dissolved in 0.1% BSA containing 0.9% saline, and VIP-RA in 0.9% saline.

Animal preparation and surgical procedures

All the surgical procedures performed for simultaneous measurements of GMBF and mean arterial blood pressure (MAP) and for drug administration were essentially as previously described in Chapter 3.2.1.2.

Experimental Protocols

After one basal measurement of GMBF and MAP, vehicle or the VIP receptor antagonist, [4Cl-D-Phe^{6},Leu^{17}]VIP (2 μmol/kg/h) was infused into the jugular vein throughout the duration of the experiment. Thirty min thereafter, vehicle or VIP (0.022 μmol/kg/h) was infused into the splenic artery at a rate of 25 μl/min for 30 min. In other studies, vehicle or [4Cl-D-Phe^{6},Leu^{17}]VIP (2 μmol/kg/h) was infused into the jugular vein throughout the duration of the experiment and 30 min after the start of the infusion, RX 77368 (30 ng) was injected intracisternally. GMBF was monitored before treatment (basal), during the 15-30 min period after the start of vehicle or [4Cl-D-Phe^{6},Leu^{17}]VIP infusion and during the 15-30 min period after VIP or RX 77368 administration in vehicle- and VIP antagonist-treated groups.

Statistics

Results are expressed as means ± SEM. Comparisons between two groups were calculated by Student's t-test. Comparisons before and after treatments were analyzed by Student's paired t-test. Multiple group comparisons were performed by ANOVA followed by Duncan's contrast. $P<0.05$ was considered statistically significant.

3.2.4.3 Results

Basal GMBF and MAP were 54.1 ± 7.3 ml/min/100 g and 71 ± 2 mmHg respectively in urethane-anesthetized rats (Fig. 19). Close intra-arterial infusion of VIP (0.022 μmol/kg/h) significantly increased GMBF to 82.1 ± 2.6 ml/min/100 g and lowered blood pressure to 60 ± 1 mmHg, while vehicle had no effect (Fig. 19). Systemic iv infusion of the VIP receptor antagonist, [4Cl-D-Phe^{6},Leu^{17}]-VIP (2 μmol/kg/h) completely abolished intra-arterial injection of VIP-induced stimulation of GMBF and hypotensive response (Fig. 19). The VIP receptor antagonist did not influence

basal levels of these parameters (Fig. 19).

In vehicle-pretreated rats, intracisternal injection of the TRH analog, RX 77368 (30 ng) stimulated GMBF (ml/min/100 g) from basal levels of 53.5 ± 3.3 to 123.6 ± 16.1, and increased blood pressure (mmHg) from basal levels of 76 ± 4 to 112 ± 5, (Fig. 20). In the presence of VIP antagonist infusion (2 μmol/kg/h), RX 77368 injected ic (30 ng) significantly stimulated GMBF to 109.6 ± 23.7 ml/min/100 g and the magnitude of the response was not significantly different compared with the vehicle-pretreated group (Fig. 20A). The VIP antagonist, which had no effect on basal MAP (75 ± 5 mmHg compared with basal 76 ± 4 mmHg), potentiated the hypertensive response to ic RX 77368 (137 ± 7 mmHg) (Fig. 20B).

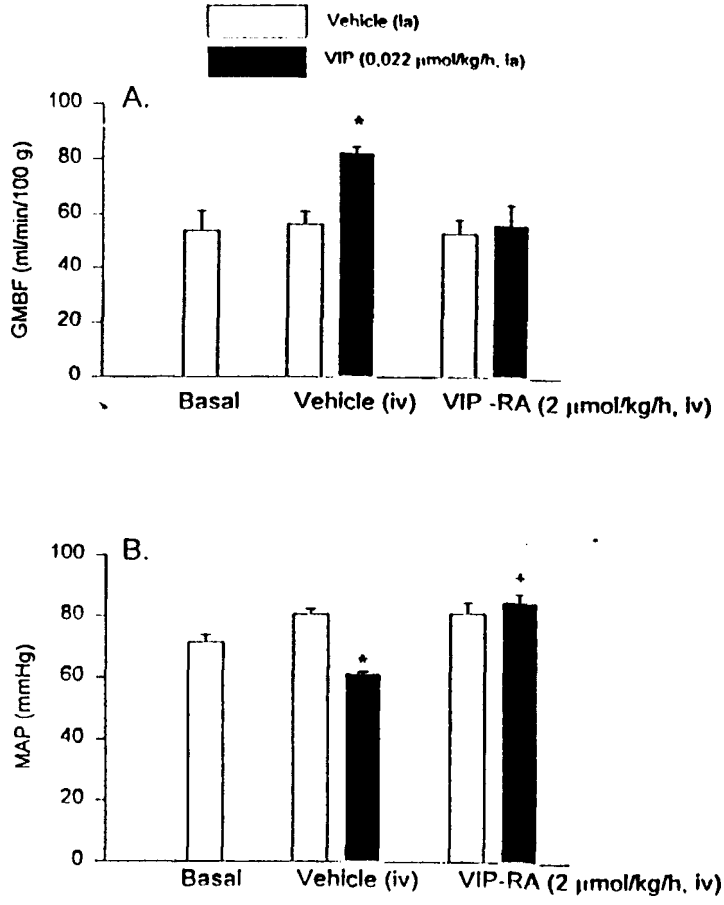

Fig. 19. Effect of VIP receptor antagonist, [4Cl-D-Phe^{6},Leu^{17}]VIP, on gastric mucosal blood (GMBF) and mean arterial pressure (MAP) changes induced by close arterial infusion of VIP in urethan-anesthetized rats. After basal measurements, vehicle or the VIP receptor antagonist was infused (2 μmol/kg/h,) intravenously and 30 min later, vehicle (25 μl/min, white columns) or VIP (0.022 μmol/kg/h) was infused into the splenic artery for 30 min. Each column represents mean ± SEM of 4-9 rats/group; * P< 0.05 compared with respective basal values; + P< 0.05 compared with vehicle + VIP treated group. MAP represents values taken at 15 min before treatment (basal), 15 min after infusion of vehicle or VIP-RA and 15 min after VIP infusion in presence of vehicle or VIP-RA treatment. GMBF values correspond to the same period.

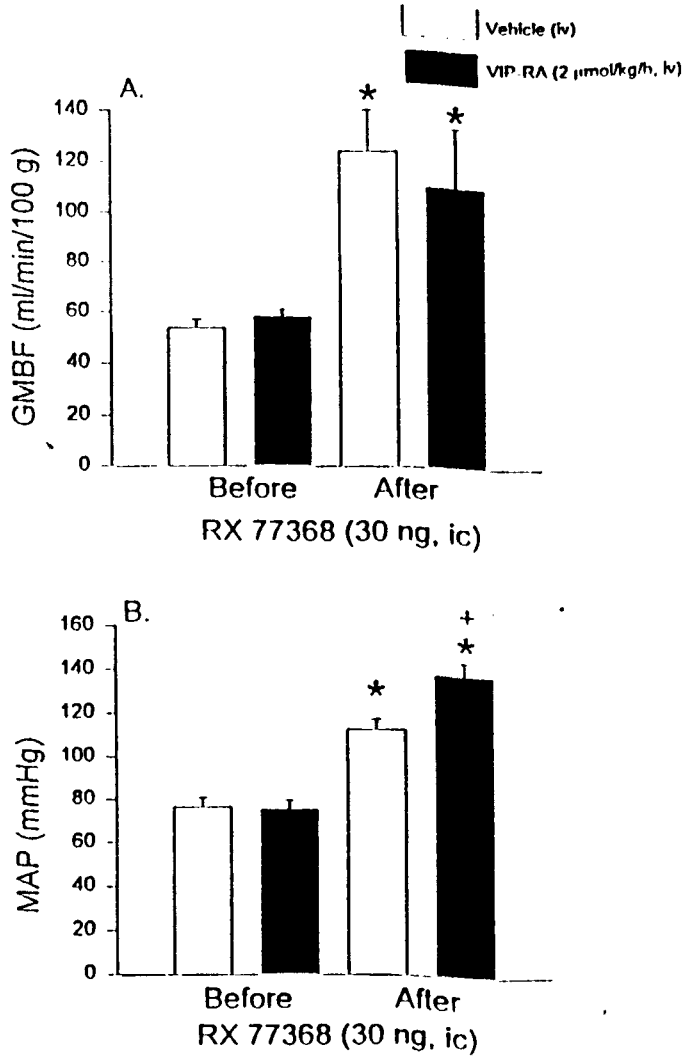

Fig. 20. Effect of VIP receptor antagonist, [4Cl-D-Phe^{6},Leu^{17}]VIP, on increase of gastric mucosal blood flow (GMBF) and mean arterial pressure (MAP) induced by intracisternal injection of RX 77368 in urethan-anesthetized rats. After a basal measurement, vehicle (1.5 ml/h, iv) or the VIP receptor antagonist (2 μmol/kg/h, iv) was infused and 30 min later, vehicle or RX 77368 was injected intracisternally. Each column represents mean ± SEM of 5-12 rats/group; * P< 0.05 compared with values before RX 77368; + P< 0.05 compared with vehicle + RX 77368 treated group. MAP represents values taken 15 min after infusion of vehicle or VIP-RA and 15 min after ic injection of RX 77368 in vehicle or VIP-RA treated groups. GMBF values correspond to the same time period.

3.2.4.4 Discussion

In the present study, close intra-arterial infusion of VIP (0.022 μmol/kg/h) increased blood flow in the gastric mucosa by 52% at a dose that lowered MAP by 11 mmHg in urethane-anesthetized rats as previously reported under similar conditions (Holzer 1991a). The rise of GMBF while there was a reduced perfusion pressure reflects an effective dilatation of gastric mucosal blood vessels in response to VIP infusion as reported in other vascular beds in rats (Bloom 1980; Ito 1987; Fahrenkrug, 1993). In dogs, intra-arterial administration of VIP also caused gastric vasodilatation (Ito 1988). The vascular responses to close intra-arterial injection of VIP namely the increase in GMBF, and decrease in MAP were completely prevented by intravenous injection of 2 μmol/kg/h of [4Cl-D-Phe^{6},Leu^{17}]VIP. The 1:100 agonist-antagonist ratio used in the present study to block VIP action is similar to that reported to be effective in other in vitro or in vivo assays (Pandol 1986; Lenz 1989). These results represent the first demonstration that [4Cl-D-Phe^{6},Leu^{17}]VIP is an antagonist for VIP-induced systemic or gastric vascular changes in the rat. Only one previous report in dogs showed the antagonistic action of [4Cl-D-Phe^{6},Leu^{17}]VIP infused into the coronary artery on VIP-induced increase in mean coronary blood flow (Quebbmann 1991). Other in vitro studies assessed the antagonist action of [4Cl-D-Phe^{6},Leu^{17}]VIP in relation VIP-induced stimulation of intestinal or pancreatic secretion or smooth muscle relaxation (Pandol 1986; Algazi 1989; Grider 1990). Recently two VIP receptor subtypes, VIP_1 and VIP_2 have been cloned from rat tissue DNA libraries (Ishihara 1992a; Lutz 1993; Usdin 1994). Pharmacological characterization revealed that [4Cl-D-Phe^{6},Leu^{17}]VIP antagonizes cAMP accumulation in cells transfected with the rat VIP_1 receptor while having no effect in cell transfected with the rat VIP_2 receptors (Rang 1991; Ishihara 1992a; Lutz 1993). Therefore, [4Cl-D-Phe^{6},Leu^{17}]VIP appears to be

more selective for the VIP_1 than the VIP_2 receptor subtype. These findings added to the more common association of VIP_1 receptor with blood vessels (Usdin 1994), suggest that exogenous VIP-induced decrease in blood pressure and increase in GMBF are mediated by an interaction with the VIP_1 receptor subtype. The absence of changes in basal MAP, and GMBF by the peripheral infusion of the VIP antagonist indicates that VIP does not participate in the maintenance of systemic and gastric vasomotor tone under basal conditions in urethane-anesthetized rats. Likewise in dogs, intracarotid infusion of [4Cl-D-Phe^6,Leu^{17}]VIP did not document a role for VIP in regulating basal coronary arterial tone (Quebbmann 1991).

Consistent reports indicate that vagal stimulation at high frequency (10 Hz) or electrical field stimulation increases VIP release from various vascular beds through atropine resistant mechanisms (Ito 1987; Ito 1988; Holst 1992; Ishihara 1992b; Quian 1996). Intracisternal injection of RX 77368 in doses as low as 1.5 ng stimulates vagal efferent discharges in urethane-anesthetized rats (O-Lee 1997). There is also evidence that VIP mediates the atropine resistant component of intracerebroventricular injection of TRH-induced vagal dependent stimulation of exocrine pancreatic and duodenal bicarbonate secretion and intestinal transport suggesting an endogenous release of VIP by central TRH (Lenz 1989; Messmer 1993; Lenz 1995). In the present study, VIP receptor blockade by systemic infusion of [4Cl-D-Phe^6,Leu^{17}]VIP enhanced the rise in MAP induced by ic RX 77368 by 25 mmHg while the VIP antagonist alone did not influence basal MAP. Previous studies indicate that RX 77368 injected ic at 30 ng or central injection of TRH increased MAP through atropine resistant mechanisms secondary to stimulation of the sympathetic nervous system (Mattila 1986; Siren 1988; Thiefin 1989; Kiraly 1998). Taken together these results suggest that endogenous VIP modulates the hypertensive response to ic RX 77368 by counteracting the increase in blood pressure owning to its

hypotensive effect . Previous studies showed that blockade of other vasodilatory substances, such as nitric oxide synthase inhibitor, L-NAME or L-NMMA further enhanced the rise in blood pressure induced to ic RX 77368 at 30 ng while calcitonin gene-related peptide antagonist, $hCGRP_{8-37}$ did alter the hypertensive response (Tanaka 1993; Kiraly 1994; Kiraly 1998). Therefore, the rise in systemic blood pressure induced by central injection of RX 77368 represents the net effect of substances released by autonomic activation having opposite modulatory influences on vascular tone.

RX 77368 injected ic at 30 ng stimulates GMBF by 2.3 fold above basal in urethane anesthetized rats as previously reported (Raybould 1990; Tanaka 1993; Kiraly 1994; Kiraly 1998). In the presence of intravenous infusion of [4Cl-D-Phe^6,Leu^{17}]VIP, RX 77368 injected ic increased GMBF by 2 fold above basal and there was no significant difference in the magnitude of the response compared with vehicle-treated group. It is unlikely that lack of significant influence of the VIP antagonist be related to the higher stimulation of GMBF induced by ic injection of RX 77368 compared with iv infusion of VIP. The VIP antagonist was used at a dose that blocked infusion of VIP at 22 nmol/kg/h. These results indicate that VIP is not the primary mediator of the increase in GMBF induced by ic RX 77368 at 30 ng. The stimulation in GMBF induced by central injection of TRH or intracisternal injection of RX 77368 at 30 ng was previously reported to be completely abolished by vagotomy and atropine (Thiefin 1989; Kiraly 1998). By contrast, VIP is a prime candidate for the non-adrenergic non-cholinergic (NANC) gastric relaxation and stimulation of blood flow in various beds observed after high frequency electrical vagal stimulation (Bloom 1980; Guth 1987; Ito 1987; Ito 1988; Thiefin 1990; Ishihara 1992b). Therefore, the stimulation of GMBF induced by ic RX 77368 in the presence of the VIP receptor antagonist is consistent with the response being primarily mediated by atropine sensitive-nitric oxide pathways

(Tanaka 1993; Kiraly 1998). Since medullary TRH plays a physiological role in the vagal efferent regulation of gastric function (Tache 1994a), it is tempting to speculate based on the present and previous data that under activation of medullary preganglionic vagal neurons, the underlying mechanisms increasing GMBF does not involved NANC VIP mediated mechanisms.

In summary, the present study shows that systemic injection of [4Cl-D-Phe^{6},Leu^{17}]VIP at 2 μmol/kg/h is an effective antagonist of VIP-induced hypotension and increase in GMBF in rats. There is no evidence for VIP being involved in the maintenance of normal resting systemic blood pressure and gastric blood flow in urethane anesthetized rats. However, upon autonomic activation by ic injection of RX 77368, endogenous VIP attenuates significantly the systemic pressor response to the central TRH analog. By contrast, VIP does not appear to play a primary role in the vagal dependent-atropine sensitive increase in GMBF induced by RX 77368 at such a dose.

3.3 Communication between vagal efferents and splanchnic sensory afferents: role of vagally activated gastric mucosal mast cells

3.3.1 Gastric hyperemia induced by intracisternal TRH analog at a cytoprotective dose is prevented by ketotifen

3.3.1.1 Background

We recently demonstrated that the gastric mucosal hyperemia and gastric protection induced by i.c. RX 77368 at 1.5 ng are mediated by vagal muscarinic dependent pathways and calcitonin gene-related peptide (CGRP) contained in capsaicin-sensitive afferent fibers (Kiraly 1997b). The mechanisms recruiting the "local effector function" of capsaicin-sensitive afferent nerve endings (Holzer, 1988; Maggi 1988) in the stomach after central activation of gastric vagal efferent discharges by RX 77368 injected i.c. at a low dose (O-Lee 1997) are still unknown.

Earlier studies indicate that electrical vagal stimulation activates mast cells in the gastrointestinal mucosa and submucosa (Cho 1977; Gottwald 1995; Santos 1996). In addition, consistent association of mast cells and neurons containing either substance P or CGRP has been demonstrated in the rat mesentery (Stead 1987; Crivellato 1991). Therefore, mast cell-derived substances known to activate capsaicin-sensitive afferent fibers (Maggi, 1991; Akoev 1996) may play an intermediary role in mediating the capsaicin and CGRP dependent gastric hyperemic response to i.c. RX 77368 at 1.5 ng. In the present study, we assessed the influence of the mast cell stabilizer, ketotifen (Grant 1990) on the increase in gastric mucosal blood flow induced by RX 773368 injected i.c. at a low dose in urethane-anesthetized rats.

3.3.1.2 Material and Methods

Drugs

RX 77368, [p-Glu-His-(3,3'-dimethyl)-Pro-NH_2; Ferring

Pharmaceuticals Ltd., Feltham, Middlesex, UK] was stored in a stock solution (3 μg/10 μl, 0.1% bovine serum albumin/saline) at -70° C. The stock solution of RX 77368 was dissolved in 0.9% saline (pH=7.0) before injection. Ketotifen (Sigma Chemical Co, St Louis, MO) was dissolved in 0.9% saline.

Animals and Surgeries

All the surgical procedures performed for simultaneous measurements of gastric mucosal blood flow, mean arterial pressure, and gastric acid secretion, as well as drug administration were essentially as previously described (Chapter 3.2.1.2) (Kiraly 1994; Kiraly 1997b).

Experimental procedures

The gastric mucosal blood flow was measured twice before (basal) and once after administration of ketotifen (2 mg/kg i.v. bolus followed by an infusion of 2 mg/kg/h throughout the experiment) or vehicle (0.3 ml followed by 1.5 ml/h infusion throughout the experiment). Thirty minutes after the start of the intravenous infusion of ketotifen or vehicle, RX 77368 (1.5 ng) was injected i.c. in 10 μl in both vehicle- and ketotifen-pretreated groups and gastric mucosal blood flow was measured for the 60-min period post injection. Mean arterial pressure values were analyzed under basal conditions, at 15 min after i.v. infusion of vehicle or ketotifen and at 15 and 45 min after i.c. injection of RX 77368. The dose of TRH analogue was based on previous experiments showing an increase in gastric mucosal blood flow through vagal capsaicin- and CGRP-dependent pathways (Kiraly 1997b). The dose of ketotifen was based on a previous *in vivo* study showing mast cell stabilizing properties (Grant 1990).

Statistics

Results are expressed as means ± S.E.M. Data were analyzed with ANOVA followed by Student-Newman-Keuls multiple comparisons

test. MAP results before and after i.c. injection of RX 77368 within the same group were also analyzed by a paired t-test. $P<0.05$ was considered statistically significant.

3.3.1.3 Results

Basal values of gastric mucosal blood flow were 52.4 ± 5.5 ml/min/100 g, mean arterial blood pressure, 70 ± 4 mmHg, gastric mucosal vascular resistance, 1.5 ± 0.2 mmHg/ml/min/100 g and gastric acid secretion, 2.2 ± 0.2 μmol/30 min in urethane anesthetized rats (Fig. 21, Table 4). Intravenous infusion of the vehicle for 15-30 min period did not modify these parameters (Fig. 21, Table 4). In vehicle-infused rats, RX 77368 injected i.c. at 1.5 ng increased gastric mucosal blood flow to 141.8 ± 18.1 ml/min/100 g ($P<0.05$) and decreased gastric mucosal vascular resistance by 59% (0.6 ± 0.1 mmHg/ml/min/100 g, $P<0.05$) as monitored during the 15-30 min period after TRH analog injection; at the 45-60 min period, these changes are returning to basal levels (Fig. 21A,B). Systemic blood pressure was increased to 82 ± 6 mmHg ($P<0.05$, paired-t-test) at 15 min after i.c. injection of RX 77368 and values were returned to basal levels at 45 min (Fig. 21C). There were no significant changes in gastric acid secretion induced by i.c. injection of RX 77368 at 1.5 ng during the 60 min period post injection (Table 4).

Ketotifen alone (2 mg/kg i.v. bolus, and 2 mg/kg/h, i.v. infusion) did not alter basal gastric mucosal blood flow, gastric vascular resistance, mean arterial pressure and gastric acid secretion as monitored during the first 15-30 min period (Fig. 21, Table 4). Ketotifen completely prevented i.c. RX 77368 at 1.5 ng-induced increase in gastric mucosal blood flow and values were maintained similar to basal level throughout the 60 min experimental period (Fig. 21A). The decrease in gastric mucosal vascular resistance induced by i.c. injection of RX 77368 at 1.5 ng was abolished by ketotifen and values (mmHg/ml/min/100 g) were

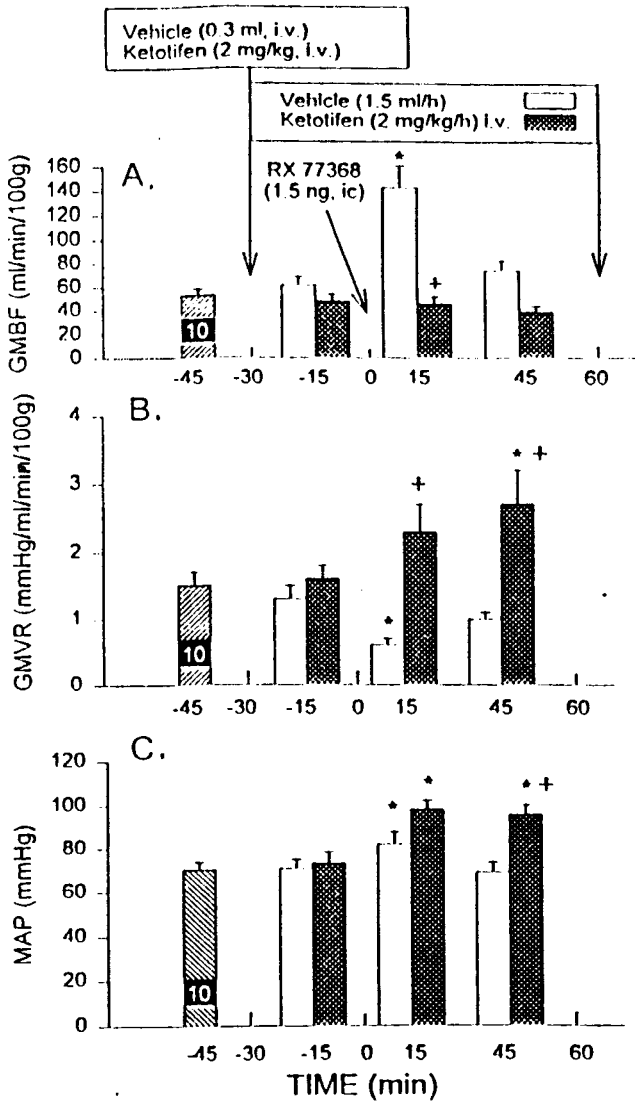

Fig. 21. Time course of the ketotifen (2 mg/kg + 2 mg/kg/h, iv, stripped columns) or vehicle (0.3 ml + 1.5 ml/h, iv, white columns) effect on RX 77368 injected intracisternally (i.c.)-induced changes in gastric mucosal blood flow, GMBF (A), gastric mucosal vascular resistance, GMVR (B), and mean arterial pressure, MAP (C) in urethane-anesthetized rats. Each column represents the mean ± S.E.M. of 4-6 rats. * P< 0.05 compared with basal levels (-45 min); + P< 0.05 compared with vehicle plus RX 77368-treated group. GMBF and GMVR values represent the 15-min period from the time indicated in min.

Table 4. Gastric acid secretion in response to i.c. injection of RX 77368 (1.5 ng) in ketotifen- or vehicle-treated urethane anesthetized rats

Treatment [a]	N	Gastric acid output (μmol/30 min)[b] Time (min)			
		Basal	0	30	60
Vehicle	6	2.3±0.5	4.3±1.7	5.1±1.5	3.0±0.7
Ketotifen	4	2.1±0.6	2.6±0.7	3.5±1.0	3.7±0.9

[a] After basal measurements, two groups of rats were injected i.v. with vehicle (0.3 ml) or ketotifen (2 mg/kg) followed by i.v. infusion of vehicle (1.5 ml/h) or ketotifen (2 mg/kg/h) for 30 min before and 60 min after i.c. injection of RX 77368 (1.5 ng.).

Acid secretion was monitored throughout the study and represents 30 min periods without treatment (basal) after vehicle or ketotifen treatment alone (0 time) and after i.c. injection of RX 77368 (30 and 60 min.)

[b] Mean ± S.E.M. of 6 and 4 rats in vehicle and ketotifen groups respectively

significantly increased (2.7 ± 0.5) at the 45-60 min period after RX 77368 injection compared with basal levels (Fig. 21B). In ketotifen-treated rats, the hypertensive response induced by i.c. injection of RX 77368 was further enhanced by 16 mmHg at 15 min post injection ($P<0.05$) and maintained significantly increased (96 ± 5 mmHg) at 45 min post injection while the mean arterial pressure in vehicle-treated group was returned to basal levels (69 ± 5 mmHg) (Fig. 21C). There was no change in gastric acid secretion in ketotifen-treated rats injected i.c. with 1.5 ng of RX 77368 (Table 4.).

3.3.1.4 Discussion

The stable TRH analog, RX 77368, injected i.c. at 1.5 ng increased gastric mucosal blood flow and systemic blood pressure while gastric mucosal resistance was decreased during the 15-30 min post injection in vehicle-pretreated, urethane-anesthetized rats. By contrast, there were no changes in gastric acid secretion for 30 min following the injection. The vascular changes induced by i.c. injection of RX 77368 at 1.5 ng are short lasting as shown by the return to basal levels at the 45-60 min period post RX 77368 injection (Kiraly 1997b), present data. Longer lasting vascular changes were observed after i.c. injection of RX 77368 at a higher dose (Tanaka 1993; Kiraly 1994). The specificity of the TRH response was previously established by the lack of vascular responses upon i.c. injection of vehicle (Kiraly 1994; Kiraly 1997b).

Previous reports indicate that the gastric hyperemic response to i.c. injection of TRH or RX 77368 is vagal-cholinergic mediated (Thiefin 1989; O-Lee 1997; Kiraly 1998). In the present study, ketotifen, administered at a dose which had no effect on basal vascular parameters, prevented i.c. RX 77368 at 1.5 ng induced-increase in gastric blood flow and decrease in gastric vascular resistance. Ketotifen is a well established mast cell stabilizer as shown by *in vivo* or *in vitro* studies in which gastrointestinal mast cell activation induced by various

treatments (toxin A, calcium ionophore, nitric oxide synthase inhibitor, substance P, and *Helicobacter pylori*) was abolished by ketotifen (Hogaboam 1993; Kubes 1993; Pothulakis 1993; Gronbech 1994; Kurose 1994). However, there is evidence in isolated intestinal smooth muscle preparations that ketotifen may also act as a non selective weak anti-muscarinic receptor antagonist (Eltze 1992) although such findings have not been confirmed (Abu-Dalu 1996). It is unlikely that the ketotifen inhibitory action observed in the present study is related to its weak antimuscarinic properties (Eltze 1992). The atropine-sensitive stimulation of other gastric functions (motility at 1.5 ng and acid secretion at 30 ng) induced by i.c. injection of RX 77368 is not modified by ketotifen under otherwise similar conditions (unpublished data). Likewise, the acid response to pylorus ligation is not altered by intragastric administration of 1-10 mg/kg of ketotifen (Karmeli 1991; Okabe 1992).

Although we did not investigate directly whether i.c. injection of RX 77368 at 1.5 ng induced a vagal cholinergic mast cell activation, convergent findings support this hypothesis. Electrical vagal stimulation decreased mast cell count in the gastric mucosa and submucosa through atropine-sensitive pathways in rats (Cho 1977; Cho 1979; Gottwald 1992). Cold exposure, known to stimulate gastric vagal activity through excitation of medullary TRH neurons (Niida 1991; Yang 1994), results in a cholinergic mediated degranulation of gastric mast cells (Cho 1979). Likewise, RX 77368 injected i.c. induced a vagal muscarinic dependent increase in the intestinal release of protease II, (Santos 1996) a specific marker for activated mucosal mast cells (Henegan 1984). Connective tissue mast cells were also shown to respond directly to a cholinergic receptor agonist (Fantozzi 1978; Masini 1985). In addition, the stomach contains a dense population of mast cells (Catto-Smith 1995) and a close anatomical relationship between mast cells, neurons and arteriolar walls has been observed in the gastric and intestinal submucosa (Newson 1983; Johnson 1992; Gronbech 1994).

Mechanisms through which ketotifen prevents the gastric hyperemic response to i.c. injection of RX 77368 at 1.5 ng are still to be established. However, there is evidence supporting the view that ketotifen action may be mediated by preventing mast cells-induced activation of capsaicin-sensitive afferent fibers. This concept is attractive since capsaicin-sensitive afferent fibers containing CGRP are closely apposed to mast cells (Stead 1987; Stead 1989; Crivellato 1991). In addition, mast cell-derived substances such as serotonin, histamine, or prostaglandin E_2 (Johnson 1992) are capable of sensitizing or activating capsaicin-sensitive fibers which are ultimately reflected by the release of CGRP (Hua 1993; Akoev 1996). Moreover, we recently showed that capsaicin pretreatment or the CGRP receptor antagonist, $hCGRP_{8-37}$ injected i.v. completely abolished i.c. RX 77368 at 1.5 ng induced gastric hyperemia (Kiraly 1997b). We also obtained preliminary electrophysiological evidence that i.c. injection of RX 77368 at 1.5 ng induces an atropine-sensitive activation of gastric splanchnic afferent fibers which is alleviated by ketotifen (O-Lee 1996). Taken together, these data suggest that the cholinergic dependent gastric hyperemia to central TRH, injected at a dose subthreshold to induce an acid secretory response, could be brought about by an interaction between vagal cholinergic stimulation and gastric mast cell response activating efferent function of capsaicin-sensitive afferent fibers to release the vasodilatory peptide, CRRP.

Central injection of TRH or TRH analog increases systemic blood pressure which is dependent upon sympathetic nervous system activation (Mattila 1986; Siren 1988; Thiefin 1989; Kiraly 1998). However, while central injection of TRH stimulates splanchnic nerve activity in the adrenal and renal branches, there is a decrease in the activity in the gastric branch (Tache 1980; Somiya 1984; Mattila 1986). In the present study, i.c. injection of RX 77368 at 1.5 ng induces a short lasting rise (11 mmHg) in systemic blood pressure as previously reported using a similar

dose of RX 77368 (Kiraly 1997b). The decrease in gastric mucosal resistance in the presence of an increase in total systemic vascular resistance indicates a local vasodilation of gastric mucosal arterioles induced by i.c. RX 77368 at 1.5 ng. In ketotifen-treated rats, the rise in mean arterial pressure induced by i.c. injection of RX 77368 was of higher magnitude (25 mmHg) and longer duration (over 45 min) and there was an increase instead of a decrease in gastric mucosal vascular resistance. Since ketotifen did not influence basal mean arterial pressure, these results indicate that mast cells alleviate the systemic hypertensive response to i.c. RX 77368 at 1.5 ng most likely through the release of vasodilatory transmitters (Hannon 1995). Ketotifen induced-blockade of gastric hyperemia is unlikely to be the consequence of the rise in systemic arterial blood pressure. Central injection of TRH or RX 77368 at higher doses induces similar hypertensive responses (28-30 mmHg) while the gastric blood flow is increased above 100% and vascular resistance is decreased (Thiefin 1989; Kiraly 1994; Kiraly 1998). In addition, atropine, vagotomy, capsaicin or CGRP receptor antagonist pretreatments abolished central TRH or RX 77368 induced increase in blood flow and decrease in vascular resistance in the gastric mucosa while the rise in systemic blood pressure was not inhibited (Thiefin 1989; Kiraly 1994; Kiraly 1997b; Kiraly 1998). The increase in gastric vascular resistance observed after RX 77368 in rats pretreated with ketotifen (present study), capsaicin and CGRP receptor antagonist (Kiraly 1997b) may reflect gastric vasoconstriction when local vasodilatory mechanisms are blocked by these treatments due to catecholaminergic influence as observed in other vascular beds after central injection of TRH (Siren 1988).

In summary, stabilization of mast cells by ketotifen abolished the gastric hyperemic response to TRH analog injected i.c. at a dose subthreshold to stimulate gastric acid secretion. These findings suggest a significant role of mast cells in the regulation of gastric mucosal blood flow in response to a low

level of central vagal activation. These observations also support the notion that mast cells, in addition to their important role in immunological and pathologic processes in the gastrointestinal tract (Crowe 1992), may participate in the physiological vagal regulation of gastric mucosal blood flow. As different stimuli may evoke different patterns of mast cell mediator release (Theoharides 1985), further studies are required to delineate the mast cell mediators involved as well as the exact mechanisms underlying the interaction between mast cells and capsaicin-sensitive afferent fibers in response to intracisternal injection of the stable TRH analog at low dose.

4. GENERAL DISCUSSIONS AND CONCLUSIONS

Considerable attention over the years has been directed toward understanding the relationship between the central nervous system and gastrointestinal functions. In particular the common association of stress-related GI mucosal damage with CNS injury has supported the hypothesis that the CNS may play a significant role in controlling the structural integrity of the GI mucosa. The establishment of the presence of family of peptide in both the gut and the brain led to speculation that these peptide may act as CNS neurotransmitters involved in the control of GI functions (Cushing, 1932; Hierlihy 1991).

Several sets of experiment suggested that intact vagal tone is essential for the maitenance of gastric mucosal integrity (Mozsik 1977; Henegan 1984; Mozsik 1993; Mozsik 1992b) and the cytoprotective effect of prostaglandins (Mozsik 1986; Cho 1992), acid inhibitors (Mozsik 1986; Cho 1992) and retinoids (Mozsik 1986).

TRH is well established to act centrally to induce vagal-dependent stimulation of gastric function in several animal species (Tache 1988; Travagli 1992; Yang 1993; Okumura 1995; Chan 1995). The dorsal motor nucleus of the vagus is the site of action of TRH or RX 77368 to stimulate gastric acid secretion, mucosal blood flow and motor function through vagal muscarinic pathways in rats and cats (Tache 1988; Hornby 1989; McCann 1989; Yang 1993; Yoneda 1993a). In addition electrophysiological studies have shown that TRH caused a direct postsynaptic excitatory effect on dorsal motor nucleus neurons (Rogers 1989; McCann 1989; Hornby 1989; Travagli 1992), and when injected into the cerebrospinal fluid, TRH increased the efferent activity in the gastric branch of the vagus (Somiya 1984).

It was recently shown that the stable TRH analog, RX 77368, injected either into the cisterna magna or into the dorsal motor nucleus of the vagus at a dose (1.5 ng) below the threshold necessary to increase gastric acid secretion prevented gastric

mucosal damage induced by ethanol via vagal cholinergic-mediated release of PGE_2 (Yoneda 1992; Yoneda 1993b).

Several findings implicated the role of endogenous nitric oxide and prostaglandins in the maintenance of the integrity of the gastric mucosa (MacNaughton 1989; Whittle 1990). The increase of the gastric mucosal blood flow has been shown to play an important role in gastric protection against injurious agents (Whittle 1990). Since there is evidence that ic TRH analog stimulates the GMBF through muscarinic and NO-dependent pathways (Tanaka 1993) we tested the hypothesis that the L-arginine-NO pathway play role in the gastric cytoprotection induced by central vagal stimulation. We demonstrated **(Chapter 3.1)** (Kiraly 1993; Tache 1994b) that NO synthase inhibitor (L-NAME) injected intravenously at à dose that inhibited nitric oxide synthesis from L-arginine (Tanaka 1993) and did not influence ethanol-induced gastric lesions, while the gastric cytoprotective effect of RX 77368 was completely abolished. The action of L-NAME was reversed by enantiomerically specific manner by L-arginine but not by D-arginine (Kiraly 1993). The protective action of nitric oxide is most likely related to its involvement in mediating the gastric hyperemic response to central vagal stimulation by TRH. Taken together these results endogenous PGE_2 and NO (Yoneda 1992; Yoneda 1993b) appear to subserve a modulator function in the vagal regulation of gastric mucosal integrity.

The crucial importance of the gastric microcirculation is thus re-enforced by the current findings that at least two chemically and physiologically distinct classes of local vasodilator mediators of diverse biochemical origins act in concert in the maintenance of mucosal viability (Whittle 1990; Holzer, 1992b). Thus we chose the measurement of gastric mucosal blood flow by using the hydrogen gas-clearance technique to further investigate the peripheral pathways of vagal nerve in the regulation of the gastric function.

RX 77368 at maximal acid secretory dose (30 ng) induced

gastric hyperemia through the activation of vagal efferent fibers. The action of TRH analogue at this dose involves vagal cholinergic activation of "local effector function" of capsaicin-sensitive afferents releasing the vasodilatory peptide CGRP **(Chapter 3.2.1)** (Kiraly 1994), whereas substance P **(Chapter 3.2.1)** (Kiraly 1994), VIP **(Chapter 3.2.4)**, histamine acting on H_1-receptor **(Chapter 3.2.2)** (Kiraly 1997c) are not the primary mediators.

In capsaicin-pretreated rats an alternate route of gastric mucosal vasodilation is activated in which nitric oxide seem to play as the final mediator **(Chapter 3.2.1)** (Fig. 18.) (Kiraly 1994; Kiraly 1998).

TRH analogue injected intracisternally at 1.5 ng (non-secretory) dose protects the gastric mucosa against ethanol injury **(Chapter 3.1)** (Yoneda 1992; Kato 1994; Kato 1995b), increases GMBF **(Chapter 3.2.3)** (Kiraly 1997b), while not stimulating gastric acid secretion **(Chapter 3.2.3)**, (Yoneda 1992; Yoneda 1993b). The gastric hyperemic response is mediated by the vasodilatory action of CGRP released from capsaicin sensitive afferent nerves. No alternate route is activated in capsaicinized rats to induce mucosal hyperemia **(Chapter 3.2.3)** (Fig. 17) (Kiraly 1997b). The lack of acid response is due to the inhibitory influence of vagally released gastric prostaglandins and CGRP **(Chapter 3.2.3)**.

Stabilization of mast cells by ketotifen prevents the hyperemic response to TRH analog injected i.c. at a cytoprotective dose suggesting a significant role of mast cells in the regulation of gastric mucosal blood flow in response to a low level of central vagal stimulation **(Chapter 3.3.1)** (Kiraly 1997a). Thus we hypothetize that mast cells might have role in the recruitment the "local effector function" of capsaicin sensitive afferent nerve endings after central activation of gastric vagal efferent fibers (Fig. 22).

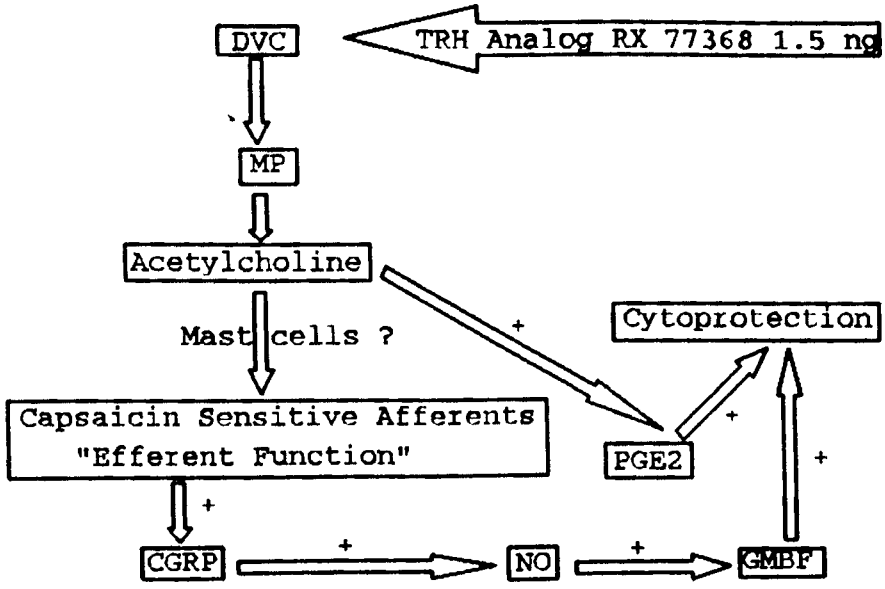

Fig. 22. Schematic representation of the action of RX 77368 injected at a cytoprotective dose (1.5 ng ic) in the dorsal vagal complex (DVC) to induce vagal muscarinic dependent stimulation of gastric secretion of histamine, prostaglandin F_2 (PGF_2) calcitonin gene-related peptide (CGRP) nitric oxide (NO) and gastric mucosal blood flow (GMBF) through the activation of mast cells

5. New Findings

1. Our study was the first providing evidence that central vagal activation induced by i.c. RX 77368 given at a cytoprotective dose increases gastric NO formation. In addition, i.v. injection of the NO precursor, L-arginine, dose-dependently prevents ethanol-induced gastric lesions whereas the D-arginine, has no effect.

2. The findings reported in gastric mucosal blood flow studies provide the first evidence to suggest that CGRP participates in the peripheral mechanisms responsible for the gastric hyperemia induced by central vagal cholinergic activation.

3. Our findings were the first suggesting that in capsaicin-treated rats, central vagal activation induced by a secretory dose of TRH analog (30 ng) increases GMBF by CGRP independent mechanisms. By contrast, in intact rats, the gastric hyperemia induced by intracisternal injection of TRH analog at the same dose involves vagal cholinergic activation of the local "effector function" of capsaicin-sensitive fibers releasing the vasodilatory peptide CGRP.

4. These data provide the very first evidence that upon central vagal activation, there is a cross talk between vagal efferent cholinergic pathways and capsaicin sensitive (splanchnic) sensory afferent fibers containing CGRP.

5. Our studies were the first showing that depending upon the level of central stimulation of the vagus alternate peripheral routes / or cascades are recruited and come into play to induce hyperemia in the mucosa.

6. The results were the first demonstration that [4Cl-D-Phe^{6},Leu^{17}]VIP is an antagonist for VIP-induced systemic or gastric vascular changes in the rat.

7. Under activation of medullary preganglionic vagal neurons, the underlying mechanisms increasing GMBF does not involved NANC VIP mediated mechanisms.

8. We provided the first functional evidence that the communication between vagal efferents and splanchnic sensory afferents is mediated by activated gastric mucosal mast cells .

6. References

Abu-Dalu, R., Zhang, J.M. and Hanani, M. The action of ketotifen on intestinal smooth muscles. *Eur J Phamacol* 309:189-193, 1996.

Akoev, G.N., Filippova, L.V. and Sherman, N.O. Mast cell mediators excite the afferents of cat small intestine. *Neuroscience* 71:1163-1166, 1996.

Algazi, M.C., Chen, H.S., Koss, M.A., et al. Effect of VIP antagonist on VIP-, PGE2-, and acid stimulated duodenal bicarbonate secretion. *Am J Physiol* 256:G833-G836, 1989.

Beattie, J. The relation of the tuber cinereum to gastric and cardiac function. *Can Med Assoc J* 26:278, 1932.

Berthoud, H.R. Morphological analysis of vagal input to gastrin releasing peptide and vasoactive intestinal peptide containing neurons in the rat glandular stomach. *J Comp Neurol* 370:61-70, 1996.

Bloom, S.R. and Edwards, A.W. Vasoactive intestinal peptide in relation to atropine resistant vasodilation in the submaxillary gland of the cat. *J Physiol* 300:41-53, 1980.

Bodis, B., Balasko, M., Csontos, Z., et al. Changes of mucosal prostaglandin contents in intact and vagotomized rats, without and with beta-carotene treatment. *Dig Dis Sci* 30:1015, 1990.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. and Macintyre, I. Calcitonin gene-related peptide is a potent vasodilator. *Nature Lond* 313:54-56, 1985.

Brown, M.R. Thyrotropin releasing factor: a putative CNS regulator of the autonomic nervous system. *Life Sci* 28:1789-1795, 1981.

Brownstein, M., Palkovits, M., Saavedra, J., Bassiri, R. and Utiger, R. Thyrotropin-releasing hormone in specific nuclei of hte rat brain. *Science* 185:267-269, 1974.

Buxton, I.L.O., Cheek, D.J., Eckman, D., Westfall, D.P., Sanders,

K.M. and Keef, K.D. N-G-nitro-L-arginine methyl ester and other alkyl esters of arginine are muscarinic receptor antagonists. *Circ Res* 72:387-395, 1993.

Catto-Smith, A.G. and Ripper, J.L. Mucosal mast cells and developmental changes in gastric absorption. *Am J Physiol* 268:G121-G127, 1995.

Chan, Y.S., Ko, J.K.S. and Cho, C.H. Role of dorsal motor nucleus of the vagus in gastric function and mucosal damage induced by ethanol in rats. *Dig Dis Sci* 40:2312-2316, 1995.

Chaudbery, T.K. and Jacobson, E.D. Prostaglandin protection of gastric mucosa. *Gastroenterology* 74:59-63, 1978.

Chen, R.Y.Z., Li, D.-S. and Guth, P.H. Role of calcitonin gene-related peptide in capsaicin-induced gastric submucosal arteriolar dilation. *Am J Physiol* 262:H1350-H1355, 1992.

Chen, R.Y.Z., Ross, G., Chyu, H.-Y. and Guth, P.H. Role of L-arginine-derived nitric oxide in cholinergic dilation of gastric arterioles. *Am J Physiol* 265:H2110-H2116, 1993.

Cho, C.H. and Ogle, C.W. The effects of zinc-sulphate on vagal-induced mast cell changes and ulcers in the rat stomach. *Eur J Pharmacol* 43:315-322, 1977.

Cho, C.H. and Ogle, C.W. Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H1- and H2-receptor activation in stress ulceration in rats. *Eur J Pharmacol* 55:23-33, 1979.

Cho, C.H., Chen, B.W., Hui, W.M., Lam, S.K. and Ogle, C.W. The role of the vagus nerve in the protective action of acid inhibitors on ethanol-induced gastric mucosal damage in rats. *J Gastroenterol Hepatol* 7:178-183, 1992.

Crivellato, E., Damiani, D., Mallardi, F. and Travan, L.

Suggestive evidence for a microanatomical relationship between mast cells and nerve fibers containing substance P, Calcitonin gene related peptide, vasoactive intestinal polypeptide, and somatostatin in the rat mesentery. *Acta Anat Basel* 141:127-131, 1991.

Crowe, S.E. and Perdue, M.H. Gastrointestinal food hypersensitivity: Basic mechanisms of pathophysiology. *Gastroenterology* 103:1075-1095, 1992.

Cushing, H. Peptic ulcers and the interbrain. *Surg Gynecol Obstet* 55:1-34, 1932.

Dennis, T., Fournier, A., Cadieux, A., et al. hCGRP8-37, a calcitonin gene-related peptide antagonist revealing calcitonin gene-related peptide receptor heterogenity in brain and periphery. *J Pharmacol Exp Ther* 254:123-128, 1990.

Eltze, M., Mutschler, E. and Lambrecht, G. Affinity profiles of pizolifen, ketotifen, and other tricyclic antimuscarinics at muscarinic receptor subtypes M1, M2 and M3. *Eur J Phamacol* 211:283-293, 1992.

Eskay, R.L., Long, R.T. and Palkovits, M. Localization of immunoreactive thyrotropin-releasing hormone in the lower brainstem of the rat. *Brain Res* 277:159-162, 1983.

Fahrenkrug, J. Transmitter role of vasoactive intestinal peptide. *Pharmacol Toxicol* 72:354-363, 1993.

Fantozzi, R., Masini, E., Blandina, P., Mannaioni, P.F. and Bani-Sacchi, T. Release of histamine of rat mast cells by acetylcholine. *Nature* 273:473-474, 1978.

Feng, H.S., Lynn, R.B., Han, J. and Brooks, F.P. Gastric effects of TRH analog and bicuculline injected into dorsal motor vagal nucleus in cats. *Am J Physiol* 259:G3210-G3216, 1990.

Forster, E.R. and Southam, E. The intrinsic and vagal extrinsic innervation of the rat stomach contains nitric oxide synthase. *NeuroRep* 4:275-278, 1993a.

Forster, E.R. and Southam, E. The intrinsic and vagal extrinsic

innervation of the rat stomach contains nitric oxide synthase. *Neurochemistry* 4:275-278, 1993b.

Garrick, T., Buack, S., Veiseh, A. and Tache, Y. Thyrotropine-releasing hormone (TRH) acts centrally to stimulate gastric contractility in rats. *Life Sci* 40:649-657, 1987.

Gates, T.S., Zimmerman, R.P., Mantyh, C.R., Vigna, S.R. and Mantyh, P.W. Calcitonin gene-related peptide-alpha receptor binding sites in the gastrointestinal tract. *Neuroscience* 31:757-770, 1989.

Gepetti, P., Tramontana, M., Evangelista, S., et al. Differencial effect on neuropeptide release of different concentrations of hydrogen ions on afferent and intrinsic neurons in the rat stomach. *Gastroenterology* 101:1505-1511, 1991.

Goto, Y. and Tache, Y. Gastric erosions induced by intracisternal thyrotropin-releasing hormone (TRH) in rats. *Peptides* 6:153-156, 1985.

Gottwald, T.P., Lhotak, S., Bienenstock, J. and Stead, R.H. Evidence for functional relationship between nerves and mucosal mast cells in rat stomach in vivo. *Gastroenterology* 102:A733, 1992.

Gottwald, T.P., Hewlett, B.R., Lhotak, S. and Stead, R.H. Electrical stimulation of the vagus nerve modulates the histamine content of mast cells in the rat jejunal mucosa. *NeuroRep* 7:313-317, 1995.

Grant, S.M., Goa, K.L., Fitton, A. and Sorkin, E.M. Ketotifen: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in asthma and allergic disorders. *Drugs* 40:412-448, 1990.

Grider, J.R. and Rivier, J.R. Vasoactive intestinal peptide (VIP) as a transmitter of inhibitory motor neurons of the gut: evidence from the use of selective VIP antagonist and VIP antiserum. *J Pharmacol Exp Ther* 253:738-742, 1990.

Gronbech, J.E. and Lacy, E.R. Substance P attenuates gastric

mucosal hyperemia after stimulation of sensory neurons in the rat stomach. *Gastroenterology* 106:440-449, 1994.

Guth, P.H. and Smith, E. Neural control of gastric mucosal blood flow in the rat. *Gastroenterology* 69:935-940, 1975.

Guth, P.H. and Leung, F.W. Physiology of the gastric circulation. In: *Physiology of the gastrointestinal tract*, edited by Johnson, L.R. New York: Raven Press, 1987, p. 1031-1053.

Guth, P.H. Pentagastrin and histamine in the regulation of gastric mucosal blood flow. *Scand J Gastroenterol* 26 (suppl180):118-125, 1991.

Hannon, J.P., Pfankuche, H.J. and Fozard, J.R. Role of mast cells in adenosine A3 receptor-mediated hypotension in the rat. *Br J Pharmacol* 115:945-952, 1995.

Henegan, J.M., Smith, G.S., Seidel, E.R. and Miller, T.A. Influence of vagotomy on mucosal protection against alkohol-induced gastric damage in the rat. *Gastroenterology* 87:903-908, 1984.

Heslop, T. The nervous control of gastric secretion. An experimental study. *Br J Surg* 25:884-899, 1938.

Hierlihy, L.E., Wallace, J.L. and Ferguson, A.V. Vagal stimulation-induced gastric damage in rats. *Am J Physiol* 261:G104-G110, 1991.

Hogaboam, C.M., Bisonette, E.Y., Chin, B.C., Befus, A.D. and Wallace, J.L. Prostaglandins inhibit inflammatory mediator release from rat mast cells. *Gastroenterology* 104:122-129, 1993.

Holm, L. and Jagere, A. Role of prostaglandinsin regulation of gastric mucosal blood flow and acid secretion. *Am J Physiol* 263:G446-G451, 1992.

Holm, L. and Jarere, A. Histamine is not involved in pentagastrin-induced gastric mucosal vasodilation in the rat. *Am J Physiol* 266:G55-G61, 1994.

Holst, J.J., Skak-Nielsen, T., Orskov, C. and Seier-Poulsen, S.

Vagal control of the release of somatostatin, vasoactive intestinal polypeptide, gastrin-releasing peptide, and HCL from porcine non-antral stomach. *Scand J Gastroenterol* 27:677-685, 1992.

Holzer, P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neurotransmitters. *Neuroscience* 24:739-768, 1988.

Holzer, P. and Guth, P.H. Neuropeptide control of rat gastric mucosal blood flow. *Circ Res* 68:100-105, 1991a.

Holzer, P., Livingston, P.E., Saria, A. and Guth, P.H. Sensory neurons mediate protective vasodilation in rat gastric mucosa. *Am J Physiol* 260:G363-G370, 1991b.

Holzer, P. Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. *Rev Physiol Biochem Pharmacol* 121:50-146, 1992a.

Holzer, P. Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. *Rev Physiol Biochem Pharmacol* 121:49-146, 1992b.

Holzer, P., Lippe, I.T., Jocic, M., Wachter, C., Erb, R. and Heinemann, A. Nitric oxide-dependent and -independent hyperemia due to calcitonin gene-related peptide in the rat stomach. *Br J Pharmacol* 110:404-410, 1993.

Hornby, P.J., Rossiter, C.D., Pineo, S.V., et al. TRH: immunocytochemical distribution in vagal nuclei of the cat and physiological effects of microinjection. *Am J Physiol* 257:G454-G462, 1989.

Hua, X.Y. and Yaksh, T.L. Pharmacology of the effects of bradykinin, serotonin, and histamine on the release of calcitonin gene-related peptide from C-fibre terminals in the rat trachea. *J Neurosci* 13(5):1947-1953, 1993.

iadecola, C.X.X., Zhang, F., Hu, J. and El-Fakahany, E.E.

Prolonged inhibition of brain nitric oxide synthase by short term systemic administreation of nitro-L-arginine methyl ester. *Neurochem Res* 19:501-505, 1994.

Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal peptide. *Neuron* 8:811-819, 1992a.

Ishihara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. Functional expression and tissue distribution of a novel receptor for vasoactive intestinal peptide. *Neuron* 8:811-819, 1992b.

Ishikawa, Y., Yang, H. and Tache, Y. Medullary sites of action of the TRH analogue, RX 77368, for stimulation of gastric acid secretion in the rat. *Gastroenterology* 95:1470-1476, 1988.

Ito, H., Matsuda, K., Sato, A. and Tohgi, H. Cholinergic and VIPergic vasodilatory actions of paresympathetic nerves on the thyroid blood flow in rats. *Jpn J Physiol* 37:1005-1017, 1987.

Ito, S., Ohga, A. and Ohta, T. Gastric vasodilatation and vasoactive intestinal peptide output in response to vagal stimulation in the dog. *J Physiol* 404:669-682, 1988.

Javor, T., Bata, M., Lovasz, L., et al. Gastric cytoprotective effect of vitamin A and other carotenoids. *Int.J.Tiss.Reac.* 5:289-296, 1983.

Johnson, D. and Krenger, W. Interactions of mast cells with the nervous system - Recent advances. *Neurochem Res* 17:939-951, 1992.

Kaneko, H., Kato, K., Ohnig, G. and Tache, Y. Medullary thyreotropin-releasing hormone mediates vagal-dependent adaptive gastric protection induced by mild acid in rats. *Gastroenterology* 109:861-865, 1995a.

Kaneko, H., Yang, H., Ohnig, G. and Tache, Y. Medullary TRH is involved in gastric protection induced by low dose of kainic acid into the raphe pallidus. *Am J Physiol* 268:G548-G552, 1995b.

Karadi, O., Bodis, B., **Kiraly, A.**, et al. Surgical vagotomy enhances the indomethacin-induced gastrointestinal mucosal damage in rats. *Inflammopharmacology* 2:349-389, 1994.

Karmeli, F., Eliakim, R., Okon, E. and Rachmilewitz, D. Gastric mucosal damage by ethanol is mediated by substance P and prevented by ketotifen, a mast cell stabilizer. *Gastroenterology* 100:1206-1216, 1991.

Kato, K., Yang, H. and Tache, Y. Role of peripheral capsaicin sensitive neurones and CGRP in the central vagally mediated gastroprotective effect of TRH. *Am J Physiol* 266:R1610-R1614, 1994.

Kato, K., Martinez, V., StPierre, S. and Tache, Y. CGRP antagonists enhance gastric acid secretion in 2-h pylorus-ligated rats. *Peptides* 16:1257-1263, 1995a.

Kato, K., Yang, H. and Tache, Y. Low doses of TRH analogue act in the dorsal motor nucleus to induce gastric protection in rats. *Am J Physiol* 269:R1301-R1307, 1995b.

Kato, K., Yang, H. and Tache, Y. Role of prostaglandins and calcitonin gene-related peptide in central vagal cholinergic-dependent protection against gastric injury in urethane-anesthetized rats. *Digestion* 57:322-327, 1996.

Kiraly, A., Suto, G., Vincze, A. and Mozsik, G. Acute and chronic surgical vagotomy (SV) and gastric mucosal vascular permeability in ethanol-treated rats. *Acta Physiol Hung* 80(1-4):219-224, 1992.

Kiraly, A., Suto, G. and Tache, Y. Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. *Eur J Phamacol* 240:299-301, 1993.

Kiraly, A., Suto, G., Livingston, P.E., Guth, P.H., St.Pierre, S. and Tache, Y. Central vagal activation by TRH induces gastric hyperemia: role of CGRP in capsaicin-sensitive afferents in rats. *Am J Physiol* 267:G1041-G1049, 1994.

Kiraly, A., Suto, G., Guth, P.H. and Tache, Y. Ketotifen prevents

gastric hyperemia induced by intracisternal thyrotropin-releasing hormone at a low dose. *Eur J Pharmacol* 334:241-247, 1997a.

Kiraly, A., Suto, G., Guth, P.H. and Tache, Y. Mechanismas mediading gastric hyperemia and acid responses to central TRH analog at a cytoprotective dose. *Am J Physiol* 273:G31-G38, 1997b.

Kiraly, A., Suto, G., Guth, P.H. and Tache, Y. Influence of [4 Cl-D-Phe6,Leu17]VIP on VIP-and central TRH-induced gastric hyperemia. *Peptides* 18(9):1321-1325, 1997c.

Kiraly, A., Suto, G., Guth, P.H. and Tache, Y. Peripheral mediators involved in the gastric hyperemic response to vagal activation by central TRH in rats. *Am J Physiol* 274:G170-G177, 1998.

Kitagawa, H., Takeda, F. and Kohei, H. Endothelium dependent increases in rat gastric mucosal hemodynamics induced by acetylcholine and vagal stimulation. *Eur J Phamacol* 133:57-63, 1987a.

Kitagawa, H., Takeda, F. and Kohei, H. Endothelium dependent increases in rat gastric mucosal hemodynamics induced by acetylcholine and vagal stimulation. *Eur J Phamacol* 133:57-63, 1987b.

Kubes, P., Kanwar, S., Niu, X-F. and Gaboury, J.P. Nitric oxide synthesis inhibition induces leukocyte adhesion via superoxide and mast cells. *FASEB J.* 7:1293-1299, 1993.

Kurose, I., Granger, D.N., Evans, D.J., et al. Helicobacter pylori-induced microvascular protein leakage in rats: Role of neutrophils, mast cells and platelets. *Gastroenterology* 107:70-79, 1994.

Lacolley, P.J., Lewis, S.J. and Brody, M.J. Role of sympathetic nerve activity in the generation of vascular nitric oxide in urethane-anesthetized rats. *Hypertension* 17:881-887, 1991.

Lambrecht, N., Burchert, M., Respondek, M., Muller, K.M. and Peskar, B.M. Role of calcitonin gene-related peptide and nitric oxide in the gastroprotective effect of capsaicin in the rat. *Gastroenterology* 104:1371-1380, 1993.

Laughton, W.B. and Powley, T.L. Localisation of efferent function in the dorsal motor nucleus of the vagus. *Am J Physiol* 252:R13-R19, 1987.

Lefebvre, R.A., Hasrat, J. and Gobert, A. Influence of N-G-nitro-L-arginine methyl ester on vagally induced gastric relaxation in the anesthetized rats. *Br J Pharmacol* 105:315-320, 1992.

Lenz, H.J., Vale, W.W. and Rivier, J.E. TRH-induced vagal stimulation of duodenal HCO3 mediated by VIP and muscarinic pathways. *Am J Physiol* 257:G677-G682, 1989.

Lenz, H.J. and Silverman, T.A. Effects cerebral TRH on intestinal water transport: role of vagal, muscarinic, and VIP pathways. *Am J Physiol* 269:G138-G143, 1995.

Lenz, J.H., Vale, W.W. and Rivier, J.E. TRH-induced vagal stimulation of duodenal HCO3 mediated by VIP and muscarinic pathways. *Am J Physiol* 257:G677-G682, 1989.

Leung, F.W., Guth, P.H., Scremin, O.U., Golanska, E.M. and Kaufmann, G.L. Regional gastric mucosal blood flow measurements by hydrogen gas clearance in the anethetized rat and rabbit. *Gastroenterology* 87:28-36, 1984.

Li, D., Raybould, H., Quintero, E. and Guth, P.H. Role of calcitonin gene-related peptide in gastric hyperemic response to intragastric capsaicin. *Am J Physiol* 261:G657-G661, 1991.

Li, D.S., Raybould, H.E., Quintero, E. and Guth, P.H. Calcitonin gene-related peptide mediates the gastric hyperemic response to acid back-diffusion. *Gastroenterology* 102:1124-1128, 1992.

Livingston, P.E., Reedy, T., Leung, F.W. and Guth, P.H. Computerized curve fitting in the analysis of hydrogen gas clearance curves. *Am J Physiol* 257:G668-G675, 1989.

Lopez-Belmonte, J., Whittle, B.J.R. and Moncada, S. The actions of nitric oxide donors in the prevention or induction of injury to the rat gastric mucosa. *Br J Pharmacol* xx:73-78, 1992.

Lundberg, J.M., Anggard, A., Fahrenkrug, J., Hokfelt, T. and Mutt, V. Vasoactive intestinal peptide in cholinergic neurons of exocrine glands: functional significance of coexisting transmitters for vasodilation and secretion. *Proc Natl Acad Sci USA* 77:1651-1655, 1980.

Lundberg, J.M., Franco-Cereceda, A., Alving, K., Delay-Goyet, P. and Lou, Y.P. Release of calcitonin gene-related peptide from sensory nerve. *Ann N Y Acad Sci* 657:187-193, 1992.

Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G. and Harmar, A.J. The VIP2 receptor: molecular characterisation of a cDNA encoding a novel receptor for vasoactive intestinal peptide. *FEBS Lett* 334:3-8, 1993.

MacNaughton, W.K., Cirino, G. and Wallace, J.L. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sci* 45:1869-1876, 1989.

Madea-Hagiwara, M. and Tache, Y. Central nervous system action of TRH to stimulate gastric emptying in rats. *Peptides* 14:199-207, 1987.

Maggi, C.A. and Meli, A. The sensory-efferent function of capsaicin sensitive sensory neurons. *Gen Pharmacol* 19:1-43, 1988.

Maggi, C.A. The pharmacology of efferent function of sensory nerves. *J Auton Pharmacol* 11:173-208, 1991.

Masini, E., Fantozzi, R., Conti, A., Blandina, P., Brunelleshi, S. and Mannaioni, P.F. Mast cell heterogenity in response to cholinergic stimulation. *Int Arch Allergy Appl Immunol* 77:184, 1985.

Mattila, J. and Bunag, R.D. Sympathomimetic pressor responses to thyrotropin-releasing hormone in rats. *Am J Physiol* 251:H86-H92, 1986.

McCann, M.J., Hermann, G.E. and Rogers, R.C. Thyrotropine-releasing hormone: effects on identified neurons of the dorsal vagal complex. *J Auton Nerv Syst* 26:107-112, 1989.

Messmer, B., Zimmerman, F.G. and Lenz, J.H. Regulation of exocrine pancreatic secretion by cerebral TRH and CGRP : role of VIP, muscarinic, and adrenergic pathways. *Am J Physiol* 264:G237-G242, 1993.

Messner, B., Zimmerman, F.G. and Lenz, H.J. Regulation of exocrine pancreatic secretion by cerebral TRH ang CGRP: role of VIP, muscarinic and adrenergic pathways. *Am J Physiol* 264:G237-G242, 1993.

Morishita, T. and Guth, P. Vagal nerve stimulation causes noncholinergic dilatation of gastric arterioles. *Am J Physiol* 250:G660-G664, 1986.

Mozsik, G. and Vizi, F. Surgical vagotomy and stomach wall ATP and ADP in pylorus ligated rats. *Amer.J.Dig.Dis.* 22:1072, 1977.

Mozsik, G., Moron, F., Nagy, L., Ruzsa, C., Tarnok, F. and Javor, T. Evidence of the gastric cytoprotective effects of vitamin A, atropine and cimetidine on the development of gastric mucosal damage induced by administration of indomethacin in healthy subjects. *Int.J.Tiss.Reac.* 8:85-90, 1986.

Mozsik, G., **Kiraly, A.**, Garamszegi, M., et al. Failure of prostacyclin, beta-carotene, atropine and cimetidine to produce gastric cyto- and general mucosal protection in surgically vagotomized rats. *Life Sci* 49:1381-1389, 1991.

Mozsik, G., **Kiraly, A.**, Garamszegi, M., et al. Mechanisms of vagal nerve in gastric mucosal defense: inchanged gastric emptying and increased vascular permeability. *J Clin Gastroenterol* 14 (Suppl.):S140-S144, 1992a.

Mozsik, G., Suto, G. and Vincze, A. Correlations between the acute chemical and surgical vagotomy-induced gastric mucosal biochemistry in rats. *J Clin Gastroenterol* 14(Suppl. 2):S135-S139, 1992b.

Mozsik, G., Karadi, O., **Kiraly, A.**, et al. Vagal nerve and gastric mucosal defense. *J.Physiol.(Paris)* 87:329, 1993.

Newson, B., Dahlstrom, A., Enerback, L. and Ahlman, H. Suggestive evidence for a direct innervation of mucosal mast cells.

Neuroscience 10:565-570, 1983.

Niida, H., Takeuchi, K., Ueshima, K. and Okabe, S. Vagally mediated acid hypersecretion and lasion formation in anethetized rat under hypothermic conditions. *Dig Dis Sci* 36:441-448, 1991.

O-Lee, T.J., Wei, J.Y. and Tache, Y. Intracisternal TRH activates splanchnic afferent discharge via cholinergic pathways. *Gastroenterology* 110:A1105, 1996.

O-Lee, T.J., Wei, J.Y. and Tache, Y. Intracisternal TRH and RX 77368 potently activate vagal efferent discharge in rats. *Peptides* 18:213-219, 1997.

Okabe, S., Nakaji, S. and Tachibana, M. Effect of ketotifen on acute gastric lesions and gastric secretion in rats. *Japan J Pharmacol* 59:251-254, 1992.

Okuma, Y., Osumi, Y., Ishikawa, T. and Mitsuma, T. Enhancement of gastric acid output and mucosal blood flow by tripeptide thyrotropin releasing hormone microinjected into the dorsal motor nucleus of the vagus in rats. *Jpn J Pharmacol* 43:173-178, 1987.

Okumura, T., Taylor, I.L., Ohnig, G., Tache, Y. and Pappas, T.N. Intracisternal injection of TRH antibody blocks gastric emptying stimulated by 2-deoxy-D-glucose in rats. *Brain Res* 674:137-141, 1995.

Palmer, R.M., Ashton, D.S. and Moncada, S. Vascular endothelial cells synthetize nitric oxide from L-arginine. *Nature* 333:664-666, 1988.

Pandol, S.J., Dharmsathaphorn, K., Schoeffield, M.S., Vale, W. and Rivier, J.E. Vasoactive intestinal peptide receptor antagonist [4Cl-D-Pge6, Leu17]VIP. *Am J Physiol* 250:G553-G557, 1986.

Pique, J.M., Whittle, B.J.R. and Espuluges, J. The vasodilator role of endogenous nitric oxide in the rat gastric microcirculation. *Eur J Phamacol* 174:293-296, 1989.

Plourde, V., Wong, H.C., Walsh, J.H., Raybould, H.E. and Tache,

Y. CGRP antagonist and capsaicin on celiac ganglia partly prevent postoperative ileus. *Peptides* 14:1225-1229, 1993.

Pothulakis, C., Karmeli, F., Kelly, C.P., et al. Ketotifen inhibits Clostridium difficile ToxinA-induced enteritis in rat ileum. *Gastroenterology* 105:701-707, 1993.

Quebbmann, B.B., Rivier, J. and Bache, R.J. Effects of intracoronary infusion of the vasoactive intestinal peptide antagonist [4-Cl-D-Phe6-Leu17]VIP in awake dog. *Peptides* 12:989-993, 1991.

Quian, B., Danielsson, A. and El-Salhy, M. Vagal regulation of gastrointestinal neuroendocrin system. *Scand J Gastroenterol* 31:529-540, 1996.

Ragenbass, M., Vozzi, C., Tribollet, E., Dubois-Dauphin, N. and Dreifuss, J.J. Thyrotropin-releasing hormone causes direct excitation of dorsal vagal and solitary tract neurons in rat brainstem slices. *Brain Res* 530:85-93, 1990.

Ralevic, V., Khalil, Z., Dusting, G.J. and Helme, R.D. Nitric oxide and sensory nerves are involved in the vasodilator response to acetylcholine but not calcitonin gene-related peptide in rat skin microvasculature. *Br J Pharmacol* 106:650-655, 1992.

Rang, H.P., Bevan, S. and Dray, A. Chemical activation of nociceptive peripheral neurones. *British Medical Bulletin* 47:534-548, 1991.

Raybould, H.E., Holzer, P., Reddy, S.N., Yang, H. and Tache, Y. Capsaicin-sensitive vagal afferents contribute to gastric acid and vascular responses to intracisternal TRH analog. *Peptides* 11:789-795, 1990.

Raybould, H.E., Sternini, C., Eysselein, V.E., Yoneda, M. and Holzer, P. Selective ablation of spinal afferent neurons containing CGRP attenuates gastric hyperemic response to acid. *Peptides* 13:249-254, 1992.

Rees, D.D., Palmer, R.M.J. and Moncada, S. Role of endothelium

derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 86:3375-3378, 1989.

Rees, D.D., Palmer, R.M.J., Schulz, R., Hodson, H.F. and Moncada, S. Characterisation of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br J Pharmacol* 101:746-752, 1990.

Rinaman, L., Card, J.P., Schwaber, J.S. and Miselis, R.R. Ultrastructural demonstration of gastric monosynaptic vagal circuit in the nucleus of the solitary tract in rat. *J.Neurosci* 9:1985-1991, 1989.

Robert, A., Hanchar, A.J., Lancaster, C. and Nezamis, J.E. Antisecretory and cytoprotective effects of prostacyclin (PGI2). *Fed Proc* 37:460, 1978.

Rogers, R. and McCann, M.J. Effects of TRH on the activity of gastric inflation -related neurons in the solitary nucleus in the rat. *Neurosci Lett* 104:71-76, 1989.

Rogers, R.C. and McCann, M.J. Effects of TRH on the activity of gastric inflation-related neurons in the solitary nucleus in the rat. *Neurosci Lett* 104:71-78, 1989.

Santos, J., Saperas, E., Mourelle, M., Antolin, M. and Malagelada, J.R. Regulation of intestinal mast cells and luminal protein release by cerebral thyrotropin releasing hormone in rats. *Gastroenterology* 111:1465-1473, 1996.

Saperas, E., Kaufmann, G.L. and Tache, Y. Role of prostaglandin E2 in the regulation of gastric acid secretion in the rat. *Eur J Phamacol* 209:1-7, 1991.

Saperas, E., Santos, J., Mourelle, M., Aguage, S. and Malagelada, J.R. Intracisternal TRH regulates mucosal immune system and intestinal permeability. *Gastroenterology* 1994.

Saperas, E., Mourelle, M., Santos, J., Moncada, S. and Malagelada, J.R. Central vagal activation by analogue of TRH stimulates gastric nitric oxide release in rats. *Am J Physiol* 268:G895-G899, 1995.

Sharley, K.A., Williams, R.G. and Dockray, G.J. Sensory substance P innervation of the stomach and pancreas: demonstration of capsaicin sensitive sensory neurons in the rat by combined immunohistochemistry and retrograde tracing. *Gastroenterology* 87:914-921, 1984.

Singh, J. Prostaglandin release from rat stomach following vagal stimulation or administration of acetylcholine. *Eur J Phamacol* 65:39-48, 1980.

Siren, A.-L., Lake, C.R. and Feuerstein, G. Hemodinamic and neural mechanisms of action of thyrotropin releasing hormone in the rat. *Circ Res* 62:139-154, 1988.

Snider, R.M., Constantine, J.W., LoweIII, J.A., et al. A potent non-peptide antagonist of the substance P (NK1) receptor. *Science Wash DC* 251:435-437, 1991.

Somiya, H. and Tonoue, T. Neuropeptides as central integrators of autonomic nerve activity: effects of TRH, SRIF, VIP and bombesin on gastric and adrenal nerves. *Regul pept* 9:47-52, 1984.

Stead, R.H., Tomioka, M., Simon, G.T., Quinonez, G., Felten, S.Y. and Bienenstock, J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 84:2975-2979, 1987.

Stead, R.H., Dixon, M.F., Bramwell N.H., and Bienenstock, J. Mast cells are closely opposed to nerves in the human gastrointestinal mucosa. *Gastroenterology* 97:575-585, 1989.

Stephens, R.L. and Tache, Y. Intracisternal injection of a TRH analogue stimulates gastric luminal serotonin release in rats. *Am J Physiol* 256:G377-G383, 1989.

Sternini, C., Reeve, J.R.J. and Becha, N. Distribution and characterisation of calcitonin gene-related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats. *Gastroenterology* 93:852-862, 1987.

Sternini, C. Enteric and visceral afferent CGRP neurons: targets

of innervation and differential expression patterns. *Ann N Y Acad Sci* 657:170-186, 1992.

Suto, G., **Kiraly, A.**, Vincze, A. and Mozsik, G. Effect of acute surgical vagotomy on the mucosal content of 6-keto-PGF1-alpha, PGE2, and glutathion after intragastric 96% ethanol treatment in rats. *Acta Physiol Hung* 80:205-211, 1992.

Swan, K.G. and Jacobson, E.D. Gastric blood flow and secretion in coscious dogs. *Am J Physiol* 212:891-896, 1967.

Tache, Y., Vale, W. and Brown, M. Thyrotropine-releasing hormone-CNS action to stimulate gastric acid secretion. *Nature* 287:149, 1980.

Tache, Y., Madea-Hagiwara, M., Goto, Y. and Garrick, T. Central nervous system action of TRH to stimulate gastric function and ulceration. *Peptides* 9:S9-S13, 1988.

Tache, Y., Garrick, T. and Raybould, H. Central nervous system action of peptides to influence gastrointestinal motor function. *Gastroenterology* 98:517-528, 1990.

Tache, Y. Inhibition of gastric acid secretion and ulcers by calcitonin gene-related peptide. *Ann N Y Acad Sci* 657:240-247, 1992.

Tache, Y., Yang, H. and Yoneda, M. Vagal regulation of gastric function involves thyrotropin-releasing hormone in the medullary raphe nuclei and dorsal vagal complex. *Digestion* 54:65-72, 1993a.

Tache, Y. and Yoneda, M. Central action of TRH to induce vagally mediated gastric cytoprotection and ulcer formation in rats. *J Clin Gastroenterol* 17(suppl.1):S58-S63, 1993b.

Tache, Y. and Yang, H. Role of medullary TRH in the vagal regulation of gastric function. In: *Innervation of the gut: pathophysiological implications*, edited by Tache, Y., Wingate, D.L. and Burks, T.S. Boca Raton: CRC Press, 1994a, p. 67-80.

Tache, Y., Yoneda, M., Kato, K., **Kiraly, A.**, Suto, G. and Kaneko,

H. Intracisternal thyreotropin-releasing hormone-induced vagally mediated gastric protection against ethanol lesions:Central and peripheral mechanisms. *J Gastroenterol Hepatol* 9:S29-S35, 1994b.

Takenaga, M., Kawasaki, H., Wada, A. and Eto, T. Calcitonin gene-related peptide mediates acetylcholine-induced endothelium-independent vasodilation in mesenteric resistance blood vessels of the rat. *Circ Res* 76:935-941, 1995.

Tanaka, S., Tache, Y., Kaneko, H., Guth, P.H. and Kaunitz, J.D. Central vagal activation increases mucus gel thickness and surface cell intracellular pH in rat stomach. *Gastroenterology* 112:409-417, 1997.

Tanaka, T., Guth, P. and Tache, Y. Role of nitric oxide in gastric hyperemia induced by central vagal stimulation. *Am J Physiol* 264:G280-G284, 1993.

Theoharides, T.C., Kops, S.K., Bondy, P.K. and Askenase, P.W. Different release of serotonin without coparable histamine under diverse conditions in the rat mast cell. *Biochem Pharmacol* 34:1389-1398, 1985.

Thiefin, G., Tache, Y., Leung, F.W. and Guth, P.H. Central nervous system action of thyrotropin-releasing hormone to increase gastric mucosal blood flow in the rat. *Gastroenterology* 97:405-411, 1989.

Thiefin, G., Raybould, H.E., Leung, F.W., Tache, Y. and Guth, P.H. Capsaicin-sensitive afferent fibers contribute to gastric mucosal blood flow responses to electrical vagal stimulation. *Am J Physiol* 259:G1037-G1043, 1990.

Togashi, H., Sakuma, I., Yoshioka, M., et al. Central nervous system action of nitric oxide in blood pressure regulation. *J Pharmacol Exp Ther* 262:343-347, 1992.

Travagli, R.A., Gillis, R.A. and Vicini, S. Effects of thyrotropine-releasing hormone on neurons in rat dorsal motor nucleus of the vagus, in vitro. *Am J Physiol* 263:G508-G517, 1992.

Usdin, T.B., Bonner, T.I. and Mezey, E. Two receptors for

vasoactive intestinal polypeptide with similar specificity and complementary distributions. *Endocrinology* 135:2662-2680, 1994.

Wang, Z.S., Lee, S.Y., Zhu, S.Z. and El-Fakahani, E.E. Differential coupling of m1, m3 and m5 muscarinic receptors to activation of neuronal nitric oxide synthase. *Pharmacology* 53:271-280, 1996.

Whittle, B.J.R., Lopez-Belmonte, J. and Moncada, S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory peptides in the rat. *Br J Pharmacol* 99:607-611, 1990.

Whittle, B.J.R. Neuronal and endothelium-derived mediators in the modulation of the gastric microcirculation: integrity in the balance. *Br J Pharmacol* 110:3-17, 1993.

Wu, W., Elde, R., Weesendorf, M.W. and Hokfelt, T. Identification of neurons expressing thyrotropin-releasing hormone receptor mRNA in spinal cord and lower brainstem of rat. *Neurosci Lett* 142:143-147, 1992.

Yanagisawa, K. and Tache, Y. Intracisternal TRH analogue RX 77368 stimulates gastric histamine release in rats. *Am J Physiol* 259:G599-G604, 1990a.

Yanagisawa, K., Yang, H., Walsh, J.H. and Tache, Y. Role of acetylcholine, histamine and gastrin in the acid response to intracisternal injection of TRH analog, RX 77368, in the rat. *Regul pept* 27:161-170, 1990b.

Yang, H., Ishikawa, T. and Tache, Y. Microinjection of TRH analogs into the raphe pallidus stimulates gastric acid secretion in the rat. *Brain Res* 531:280-285, 1990.

Yang, H., Stephens, R.L. and Tache, Y. TRH analogue microinjected into specific medullary nuclei stimulates gastric serotonin secretion in rats. *Am J Physiol* 262:G216-G222, 1992.

Yang, H., Ohnig, G. and Tache, Y. TRH in dorsal vagal complex mediates acid response to excitation of raphe pallidus neurons in rats. *Am J Physiol* 265:G880-G886, 1993.

Yang, H., Wu, S.V., Ishikawa, T. and Tache, Y. Cold exposure elevates thyrotropin releasing hormone gene expression in medullary raphe nuclei: Relationship with vagally mediated gastric erosions. *Neuroscience* 61:655-663, 1994.

Yoneda, M. and Tache, Y. Central thyrotropin-releasing factor analog prevents ethanol-induced gastric damage through prostaglandins in rats. *Gastroenterology* 102:1568-1574, 1992.

Yoneda, M. and Tache, Y. Potentiating interactions between medullary serotonin and thyrotropin-releasing hormone-induced gastric erosions in rats. *Neurosci Lett* 161:199-202, 1993a.

Yoneda, M. and Tache, Y. Vagal regulation of gastric prostaglandin E2 release by central TRH in rats. *Am J Physiol* 264:G231-G236, 1993b.

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Abstracts:

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1., **Á. Király**, M. Garamszegi, J. Rinfel, G. Sütö, Á. Vincze, Gy. Tóth, T. Jávor, Gy. Mózsik: Role of vagotomy on gastric cytoprotection induced by different cytoprotectiv agents. In proceedings of the 31st Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1989.

2., G. Sütö, M. Garamszegi, **Á. Király**, J.Rinfel, Á. Vincze, Gy. Tóth, T. Jávor, Gy. Mózsik: The role of adrenal cortex in the cytoprotection induced by beta-carotene during the development of gastric mucosal damage induced by 96% ethanol. In proceedings of the 31st Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1989.

3., Á. Vincze, M. Garamszegi, **Á. Király**, J. Rinfel, G. Sütő, Gy. Tóth, T. Jávor, Gy. Mózsik: Time dependent correlation between gastric cytoprotection induced by beta-carotene and the gastric mucosal vascular permeability. In proceedings of the 31st Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1989.

4., G. Sütö, **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, Á. Vincze, T. Tóth,, Gy. Mózsik: Neural and hormonal influences on beta-carotene-induced gastric mucosal cytoprotection in rats. In proceedings of the 3rd Interscience World Conference on Inflammation, Monte-Carlo, 1989.

5., Gy. Mózsik, **Á. Király**, M. Garamszegi, G. Sütö, L. Nagy, Gy. Tóth, Á. Vincze, T. Jávor: Cytoprotection and anti-ulcer effects of vitamin A and beta-carotene in the stomach. In proceedings of the 198th ACS National Meeting, Miami Beach, 1989.

6., Gy. Mózsik, **Á. Király**, M. Garamszegi, L. Nagy, G. Sütö, Gy. Tóth, Á. Vincze, T. Jávor: Vagus in the gastric mucosal injury and prevention. Dig.Dis.Sci. 34:1319, 1989.

1990

7., **Á. Király**, M. Balaskó, B. Bódis, Zs. Csontos, M. Garamszegi, O. Karádi, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy on the changes of vascular permeability during the development of gastric mucosal damage induced by 0.6 N HCl. In proceedings of the 32nd Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1990.

8., O. Karádi, M. Balaskó, B. Bódis, Zs. Csontos, M. Garamszegi, **Á. Király**, G. Sütö, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy on the development of indomethacin-induced gastric mucosal damage. In proceedings of the 32nd Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1990.

9., B. Bódis, M. Balaskó, Zs. Csontos, M. Garamszegi, O. Karádi, **Á. Király**, G. Sütö, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy on the changes of PGE2and PGI2 levels in the gastric mucosa.In proceedings of the 32nd Annual Conference of the Hungarian Gastroenterological Society,Balatonaliga, 1990.

10., G. Sütő, M. Balaskó, B. Bódis, Zs. Csontos, M. Garamszegi, O. Karádi, **Á. Király**, Á. Vincze, T. Jávor, Gy. Mózsik: Role of sulfhydryl-containing groups in beta-carotene-induced gastric cytoprotection in rats.In proceedings of the 32nd Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1990.

11., Á. Vincze, B. Bódis, M. Garamszegi, T. Jávor, **Á. Király**, O. Karádi, L. Nagy, G. Sütö, Gy. Mózsik: Correlations between the cytoprotection induced by beta-carotene and prostaglandins in intact and vagotomized rats.In proceeding of the 32nd Annual Conference of the Hungarian Gastroenterological Society, Balatonaliga, 1990.

12., **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, G. Sütö, Á. Vincze, Gy. Mózsik: Increased vascular permeability does not represent obligatory the presence of gastric hypoxia in the rat gastric mucosa in rats with intact vagus or after acute surgical vagotomy. International Symposium on Defence Mechanisms of the Digestive System. Abstract Book, p.53 Prague, 1990.

13., B. Bódis, O. Karádi, **Á. Király**, G. Sütö, Á. Vincze, Gy. Mózsik: Acute surgical vagotomy aggravates the development of gastric mucosal damage produced by ethanol, HCl and indomethacin in rats. International Symposium on Defense Mechanisms of the Digestive System. Abstract Book, p.27 Prague, 1990.

14., Gy. Mózsik, **Á. Király**, M. Garamszegi, L.Nagy, G.Sütö, Gy. Tóth, T.Jávor, Gy. Mózsik: Acute surgical vagotomy (ASV) causes an increased vascular permeability to chemicals in the rat stomach. Dig. Dis. Sci., 35:1019, 1990.

15., G. Sütö, **Á. Király**, Gy. Tóth, Á. Vincze, T. Jávor, Gy. Mózsik: Comparative study on the cytoprotection induced by PGI2, beta- -carotene and l-cysteine in experimental ulcer in rats. Dig.Dis.Sci., 35:1041, 1990.

16., Á. Vincze, M. Garamszegi, **Á. Király**, G. Sütő, Gy. Tóth, Á. Vincze, T. Jávor, Gy. Mózsik: The prevention of increased vascular permeability is not involved in gastric cytoprotective effect of beta-carotene. Dig.Dis.Sci., 35:1043, 1990.

17., **Á. Király**, M. Garamszegi, T. Jávor, G. Sütő, Á. Vincze, Gy. Tóth, Gy. Mózsik: Synergic effect of beta-carotene and selenium Dig.Dis.Sci., 35:1033, 1990.

18., B. Bódis, M. Balaskó, Zs. Csontos, O. Karádi, **Á. Király**, T. Jávor, Gy. Mózsik: Changes of gastric mucosal prostaglandin contents in intact and vagotomized rats, without and with beta-carotene treatment. Dig.Dis.Sci., 35:1015, 1990.

19., **Á. Király**, M. Balaskó, B. Bódis, Zs. Csontos, O. Karádi, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Acute surgical vagotomy (ASV) causes an increased vascular permeability to chemicals in the rat stomach. Dig.Dis.Sci., 35:1019, 1990.

20., M. Balaskó, B. Bódis, Zs. Csontos, O. Karádi, **Á. Király**, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Vagus and gastrointestinal defence in rats treated with indomethacin. Dig.Dis.Sci., 35:1015, 1990.

21., G. Sütő, **Á. Király**, Gy. Tóth, Á. Vincze, T. Jávor, Gy. Mózsik: Role of endogenous and exogenous sulfhydrils in beta-carotene-induced cytoprotection. Exp.Clin.Gastroenterol. 1:6, 1990.

22., Á. Vincze, M. Garamszegi, **Á. Király**, G. Sütő, Gy. Tóth, T. Jávor, Gy. Mózsik: The beta-carotene-induced gastric cytoprotection does not depend on the gastric mucosal prostaglandin synthesis and the microvascular injury. Exp.Clin.Gastroenterol. 1:7, 1990.

23., Gy. Mózsik, **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, M. Németh, G. Sütő, Á. Vincze: Gastric emptying and vascular permeability of the gastric mucosa in ethanol-treated rats with intact vagal nerve and after surgical vagotomy. In proceedings of the 3rd Taisho International Symposium on Gastroenterology.

Tokyo, 1990.

1991

24., **Á. Király**, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy (ASV) on the changes of gastric mucosal vascular permeability and PGE2 and 6-keto-PGF(1 alpha) during the development of 96% ethanol-induced gastric mucosal lesions. Exp. Clin.Gastroenterol. 1:8, 1991.

25., **Á. Király**, G. Sütő, Gy. Tóth, Á. Vincze, T. Jávor, Gy. Mózsik: Synergism between beta-carotene and selenium in prevention of gastric mucosal damage induced by 96% ethanol (ETOH) in rats. In proccedings of Role of free radicals in biological systems. Balatonaliga, 1991.

26., G. Sütő, **Á. Király**, Gy. Tóth, Á. Vincze, T. Jávor, Gy. Mózsik: The role of sulfhydryl groups in the development of beta-carotene-induced cytoprotection in rats. In proccedings of Role of free radicals in biological systems. Balatonaliga, 1991.

27., Gy. Mózsik, T.Jávor, **Á. Király**, G. Sütő, Á. Vincze: Correlation between the non-sulfhydryl antioxidants and prostaglandins in the gastric mucosa during its prevention. In proccedings of Role of free radicals in biological systems. Balatonaliga, 1991.

28., **Á. Király**, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy (ASV) on the changes of vascular permeability and glutathione (GSH) contents of the gastric mucosa. Z. Gastroenterol,29:190, 1991.

29., G. Sütő, **Á. Király**, T. Jávor, Á. Vincze, Gy. Mózsik: New computer assisted method to determine the area of gastric mucosal lesions induced by chemicals in rats. Z. Gastroenterol, 29:205, 1991.

30., Á. Vincze, **Á. Király**, G. Sütő, Gy. Tóth, T. Jávor, Gy. Mózsik: Biochemical and microvascular changes during beta-carotene-induced cytoprotection. Z. Gastroenterol. 29:212, 1991.

31., **Á. Király**, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy (ASV) and the changes of vascular permeability and glutathione (GSH) contents of the gastric mucosa during the development of gastric mucosal damage induced by ethanol. Digestion 49/S1:39, 1991.

32., Gy. Mózsik, T. Jávor, **Á. Király**, G. Sütö, Á. Vincze: Beta-carotene-induced gastric mucosal protection is independent of gastric mucosal levels of prostaglandins in indomethacin-treated rats with intact vagal nerve. Digestion 49/S1:46, 1991.

33., G. Sütő, B. Bódis, Zs. Csontos, O. Karádi, **Á. Király**, M. Balaskó, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of graded doses of epinephrine on the ethanol-induced gastric mucosal damage of rats. Digestion 49/S1:53, 1991.

34., Á. Vincze, **Á. Király**, G. Sütő, Gy. Tóth, T. Jávor, Gy. Mózsik: Correlations between non-protein sulfhydryls, cyclic AMP, prostaglandins, and vascular permeability during beta-carotene-induced gastric cytoprotection in ethanol model of rats. Digestion 49/S1:55, 1991.

1993

35., **Á. Király**, G. Sütő, Y. Taché: Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. Gastroenterology, 104:A455, 1993.

36., G. Sütő, **Á. Király**, V. Plourde, Y. Taché: Potent inhibition of gastric emptying by centrally and peripherally administered

interleukin-1ß in rats. Gastroenterology, 104:A523, 1993.

1994

37., **Á. Király**, G. Sütő, E. H. Livingston, P. H. Guth, Y. Taché: Peripheral mechanisms involved in gastric hyperemic response to central vagal stimulation in rats. Gastroenterology, 106:A107, 1994.

38., **Á. Király**, G. Sütő, P.H. Guth Y. Taché: Vagally mediated gastric mucosal hyperemic response to a cytoprotective dose of intracisternal TRH analog: involvment of capsaicin sensitive afferents containing CGRP and mucosal mast cells in the rat. Gastroenterology, 106:A2484, 1994.

39., G. Sütő, **Á. Király**, Y. Taché: Non-neural calcitonin gene related peptide contributes to the inhibition of gastric emptying by intraperitoneal endotoxin in rats. Gastroenterology, 106:A387, 1994.

40., G. Sütő, **Á. Király**, Y. Taché: Inhibition of gastric emptying induced by interleukin-1ß involves hypothalamic CRF release in rats. Gastroenterology, 106:A386, 1994

41. G. Sütő, **Á. Király**, Y. Taché: Laparotomy-induced inhibition is mediated by medullary release of oxytocin in rats. Gastroenterology, 106:A388, 1994.

1995

42.G. Sütő, **A. Király**, Y. Taché: Endotoxin induced inhibition of gastric emptying involves peripheral IL-1ß and CRF in rats. Gastroenterology 108: A467, 1995.

1990

1., Gy. Mózsik, **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, A. Németh, G. Sütö. Á. Vincze:Gastric cytoprotection mediating in SH-groups is failed by surgical vagotomy. **Acta Physiol.Hung.** 75(Suppl) 219, 1990.

1991

2., Gy. Mózsik, **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, G. Sütő, Gy. Tóth, Á. Vincze: Failure of prostacyclin, beta-carotene, atropine, and cimetidine to produce gastric cyto- and general mucosal protection in surgically vagotomized rats. **Life Sci.** 49:1383-1389, 1991.

1992

3., Gy. Mózsik, **Á. Király**, M. Garamszegi, T. Jávor, L. Nagy, A. Németh, G. Sütő, Á. Vincze: Mechanisms of vagus nerve in the gastric mucosal defences: unchanged gastric emptying and increased vascular permeability. **J. Clin. Gastroenterol.** 14 (Suppl. 1)S140-144, 1992

4., **Á. Király**, G. Sütő, Á. Vincze, T. Jávor, Gy. Mózsik: Acute and chronic surgical vagotomy (SV) and gastric mucosal vascular permeability in ethanol treated rats. **Acta Physiol. Hung.** 80(1-4):219-224, 1992

5., **Á. Király**, Z. Matus, G. Sütő, Á. Vincze, Gy. Mózsik: Correlation between the cytoprotective effect of ß-carotene and its gastric mucosal level in indomethacin-treated rats with or without surgical vagotomy. **Acta Physol. Hung.** 80(1-4):213-218, 1992

6., G. Sütő, **Á. Király**, Á. Vincze, T. Jávor, Gy. Mózsik: Effect of acute surgical vagotomy on the mucosal content of 6-keto-$PGF_{1\alpha}$, PGE_2 and glutathione after intragastric 96% ethanol treatment in rats. **Acta Physiol. Hung.** 80(1-4):205-212, 1992

7., Á. Vincze, Á. Király, G. Sütő, Gy. Mózsik: Is acute surgical vagotomy an aggressor to gastric mucosa in pylorus ligated rats with or without indomethacin treatment? **Acta. Physiol. Hung.** 80(1-4):195-204, 1992

8., Gy. Mózsik, Á. Király, G. Sütő, Á. Vincze: ATP breakdown and resynthesis in the development of gastrointestinal mucosal damage and its prevention in animals and human (An overwiev of 25 years ulcer research studies). **Acta Physiol. Hung.** 80(1-4):39-80, 1992

1993

9., Á. Király, G. Sütő, Y. Taché: Role of nitric oxide in the gastric cytoprotection induced by central vagal stimulation. **Eur. J. Pharmacol.** 240:299-301, 1993

1994

10., O. Karádi, Bódis B., A. Király, O.M.E.Abdel-Salam, G. Sütő, A. Vincze, Gy. Mózsik: Surgical vagotomy enhances the indomethacin-induced gastrointestinal mucosal damage in rats. **Inflammopharmacology** 2: 389-399, 1994.

11., G. Sütő, Á. Király, Y. Taché: Interleukin-1ß acts in the brain to inhibit gastric emptying in rats: Mediation through prostaglandin and CRF. **Gastroenterology** 106:1568-1575, 1994

12., Á. Király, G. Sütő, E. H. Livingston, P. H. Guth, E. St. Pierre, Y. Taché: Central vagal activation-induced increase of gastric mucosal blood flow involves CGRP in capsaicin sentive afferents in rats. **Amer. J. Physiol.** 267 (Gastrointestinal and liver physiology):G1041-1049, 1994

13., Y. Taché, M. Yoneda, K. Kato, Á. Király, G. Sütő, H. Kaneko: Intracisternal TRH induced vagally mediated gastric protection against ethanol lesions: central and peripheral mechanisms. J.

Gastroenterol. and Hepatol. 9:S29-35, 1994

1996

14., G. Sütő, **A. Király,** V. Plourde, Y. Taché: Intravenous Interleukin-1-Beta-induced inhibition of gastric emptying: involvement of central corticotrophin-releasing factor and prostaglandin pathways in rats **Digestion** 57:135-140, 1996

1997

15., **Á. Király**, G. Sütő, P.H. Guth, Y. Tache: Mechanisms mediating gastric hyperemic and acid responses to central TRH analog at a cytoprotective dose. **Am. J. Physiol.** (Gastrointestinal and Liver Physiol.)273:G31-G38, 1997.

16., **Á. Király**, J. Rivier, G. Sütő, Y. Taché: VIP and central TRH-induced gastric vasodilation: influence of [4Cl-D-Phe^{6},Leu^{17}]VIP antagonist. **Peptides** 18(9):1321-1325, 1997.

17., **A. Király**, G. Sütő, P.H. Guth, Y. Taché: Ketotifen prevents gastric hyperemia induced by intracisternal thyrotropin-releasing hormone at a low dose. **Eur. J. Pharmacol.** 334:241-247, 1997.

1998

18., **Á. Király**, G. Sütő, P. H. Guth, Y. Taché: Peripheral mediators involved in the gastric hyperemic response to vagal activation by central TRH in rats. **Am. J. Physiol.** (Gastrointestinal and Liver Physiol.) 274:G170-G177, 1998

Book chapters:

1990

1., Gy. Mózsik, **Á. Király**, M. Garamszegi, L. Nagy, G. Sütő, Gy. Tóth, Á. Vincze, T. Jávor: Role of vagus nerve in the development of gastric mucosal injury and its prevention by atropine, cimetidine, beta-carotene and prostacyclin in rats. In: S.Szabo (ed): Neuroendocrinology of gastrointestinal ulceration, Springer-Verlag Publishers, New York, pp. 264-282, 1990

1993

2., Gy. Mózsik, O. Karádi, **A. Király** , Z. Matus, G. Sütő, Á. Vincze , Gy. Tóth: Retinoids as scavengers and gastric cytoprotection in animals, human beings and patients with peptic ulcer. In Oxygen`free radicals and scavengers in the natural sciences. Gy. Mózsik, I. Emerit, J. Fehér, B. Matkovics, Á. Vincze (Eds.). Akadémiai Kiadó, Budapest, pp.329-338, 1993.

Books:

1997

1. Gy. Mózsik, L. Nagy, **A. Király** (Eds): Twenty five years of peptic ulcer research in Hungary. From basic sciences to clinical practice (1971-1995), Akadémiai Kiadó, Budapest, 1997