REGULATORY MECHANISMS OF GASTRIC SECRETION AND MOTILITY. ROLE OF CYTOKINES AND CRF PEPTIDE FAMILY AND RECEPTORS

Ph.D. thesis

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Pécs, 2006

Preface

Hans Selye pioneered the **concept of biological stress** borrowing the term from physics where "stress" stands for the interaction between a deforming force and the resistance to it. His initial reports in 1936 positioned the adrenal cortex and the gut at the center of the stress syndrome and established the role of the pituitary in the adrenal hypertrophic response to various stressful stimuli. The concept of hypothalamic neurohumoral control of pituitary secretion was validated in 1955 demonstrating the existence of **corticotropin-releasing factor** (**CRF**) that elicited adrenocorticotropin (ACTH) release from the pituitary in intact rats. The physiological importance of CRF expanded quickly, experimental studies established that CRF injected into the brain had stress-inducing properties generating the overall behavioral, **autonomic**, **immune** and **visceral responses** that were largely independent from the pituitary-adrenal stimulation. These findings paved the way to a number of investigations delineating central and peripheral sites of action, autonomic effectors along with physiological relevance of CRF signaling pathways in the adaptive response of the gut to stress. Later a great number of investigations on immunological challenges provided information about key role of cytokines in regulation of GI functions, generating the concept of **immune-brain-gut axis** (1992).

In this thesis I introduce two groups of experiments dealing with the regulation of gastric functions (secretion and motility) and role of stress mediators in these processes and review literature background related to these fields. Because of thematic considerations, I divided the thesis into two main parts. The first part details studies on the regulation of gastric motility and role of CRF agonists and interleukin-1β in the process. The second part introduces experiments on the regulation of gastric secretory function and effects of interferon-α in it.

Part I. Regulatory function of CRF system and interleukin-1 on gastric motility. Role of CRF subtype 1 and 2 receptors

BACKGROUND

CRF is a well-conserved 41-amino acid polypeptide with an identical primary structure in humans, primates, dogs, horses and rodents. In the 1980s two novel non-mammalian CRF-like peptides were discovered in non-mammalian vertebrates, namely **sauvagine** and **urotensin-I**. The cloning of CRF peptides in these species raised the question

for possible additional CRF family members in mammals, that led to the characterization of **urocortin 1** (**Ucn 1**). CRF ligands interact with CRF_1 and/or CRF_2 receptors, both belong to class B1 subfamily of seven-transmembrane domain receptors that signal largely by coupling to G_s-adenylate cyclase and differ considerably in their binding characteristics to several natural CRF ligands. The mismatches between brain distribution of CRF ligands (CRF and Ucn 1) and CRF receptors kindled search for additional endogenous CRF receptor ligands. Screening in *human and mouse genome databases* for sequence homology with the CRF/Ucn 1 family of peptides culminated in the cloning of two novel putative urocortin isoforms, **mouse urocortin 2 (mUcn 2** or **stresscopin-related peptide=SRP),** and **mouse urocortin 3 (mUcn 3** or **stresscopin).** Human isoform, human urocortin 2 (hUcn 2) lacks the consensus proteolytic cleavage site allowing for C-terminal processing so the predicted 38-amino acid mature hUcn 2 and hUcn 3 peptides have been assigned based on the structures of precursor genes and used for experiments.

CRF_1 receptor shows no appreciable binding to **Ucn 2** and **Ucn 3**, but binds with high affinity to **Ucn 1**, **CRF** and **sauvagine**. In contrast, although CRF is 100-fold more potent at the CRF_1 compared with the CRF_2 receptor, there are no natural agonists with similar selectivity for CRF_1 as Ucn 2 and Ucn 3 for CRF_2 receptors.

Key to the assessment of the role of CRF receptors in the stress response was the development of specific **CRF receptor antagonists**. Earlier studies relied on the use of non-selective CRF_1/CRF_2 receptor peptide antagonists, mainly **α-helical $CRF_{9\text{-}41}$**, **D-Phe^{12} $CRF_{12\text{-}41}$**, followed by the potent and long acting competitive antagonists, **astressin** and **astressin-B**. Recently, selective peptide **CRF_2 receptor antagonists**, namely **antisauvagine-30**, **K41498, [D-Phe^{11},His^{12},Nle^{17}]$sauvagine_{11\text{-}40}$** and the more potent, longer acting analog, **$astressin_2$-B**, were developed and characterized. **These peptide antagonists generally display a poor penetrance to the brain,** however in contrast, all **CRF_1 antagonists** have been designed as small hydrophobic molecules that **cross the blood-brain barrier**, including the first developed **CP-154,526**. These compounds have been instrumental in establishing the role of brain CRF-CRF_1 receptor signaling pathways in stress-related activation of the *hypophysis-pituitary-adrenal (HPA) axis* and *anxiogenic behavior*.

The **central action of CRF** to inhibit gastric transit has been well established under various experimental conditions. In addition to CRF, other non-mammalian and mammalian CRF-related peptides, namely **sauvagine**, **urotensin I**, **Ucn 1**, injected icv or ic **inhibit gastric emptying** of liquid or solid meal. Convergent evidences indicate that **CRF_2 receptor subtype mediates the delayed gastric emptying** induced by central injection of CRF and

related peptides in rodent, however central CRF signaling pathways are not involved in basal and postprandial regulation of gastric emptying.

The interleukin (IL)-1 system consists of the agonist ligands, IL-1α and IL-1β, the antagonist ligand, IL-1 receptor antagonist (IL-1ra) and IL-1 receptors. **IL-1β** is a 17,500-dalton cytokine that is an important mediator involved in inflammation and immune responses to stress. It is released from activated macrophages, T-lymphocytes and in the brain by microglial cells, microvessel endothelium and to a lesser degree by astrocytes.

Peripherally administered IL-1β has been shown to delay gastric emptying of solid meals as well as semi-liquid, non-nutrient meals, and to inhibit gastric acid secretion in rats. Furthermore, central or peripheral administration of IL-1β activates corticotropin-releasing factor (CRF) containing neurons in the paraventricular nucleus of the hypothalamus (PVN).

THE AIMS OF THESE SETS OF EXPERIMENTAL STUDIES

1. to characterize the central action of Ucn 2 on gastric emptying in conscious rats;
2. to compare Ucn 2 action with that of CRF_1/CRF_2 agonists, Ucn 1 or CRF, using the selective CRF_2 antagonist, $astressin_2$-B.;
3. to characterize autonomic pathways through which ic and icv Ucn 2 and ic Ucn 1 inhibit gastric emptying, using surgical (subdiaphragmatic vagotomy) and pharmacological (noradrenergic, α_1, α_2 and β adrenergic receptor blockade) approaches;
4. to investigate the role of peripheral CRF_1 and CRF_2 receptor pathways in peripheral IL-1β-induced delay of gastric emptying of a non-caloric viscous meal in rats using the selective CRF_1 antagonist, CP-154,526 and the selective CRF_2 antagonist, $astressin_2$-B;
5. to investigate the role of central CRF_1 and CRF_2 receptor pathways in peripheral IL-1β-induced delay of gastric emptying of a non-caloric viscous meal in rats using the selective CRF_1 antagonist, CP-154,526 and the selective CRF_2 antagonist, $astressin_2$-B.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing 250-300 g were housed in group cages under controlled illumination, humidity and temperature, and had free access to tap water and Purina® rat chow. Rats were deprived of food in wired-bottom fasting cages, but had free access to tap water for 16-18 h before the experiments, except as otherwise stated.

Peptides, drugs and substances

R/h CRF, **r/h Ucn 1** and **hUcn 2** were dissolved in saline and **astressin$_2$-B** in double-distilled sterile water (pH 7.0) right before use. **Bretylium tosylate, propranolol hydrochloride** and **prazosin hydrochloride** were dissolved in sterile saline just before experiments, while **yohimbine hydrochloride** was dissolved in 5% dimethyl-sulfoxide (DMSO) and 95 % distilled water. **Phenol red** and **methylcellulose** viscouse dye was prepared on the day of experiments. **Ketamine hydrochloride** and **xylazine** were used to anesthetize rats undergoing vagotomy or intracerebroventricular cannula implantation as stated later. The non-peptide selective CRF_1 antagonist, **CP-154,526** was dissolved in 10% DMSO, 10% polyoxyethylene sorbitan monooleate and 80% sterile saline when injected subcutaneously (sc), and it was dissolved in DMSO only when injected intracerebroventicularly (icv). While CP-154,526 has low pH (pH equals 2.0 approximately), pH of its vechicle was matched to this pH by addition of HCl solution in cotrol groups. **Recombinant human interleukin-1β** was dissolved in 0.1% bovine albumin right before use.

Treatments and measurements

Intracisternal injections (ic) were performed under short isoflurane anesthesia (2-3 min, 5% vapor concentration in oxygen) by puncturing occipital membrane with a 50 µl Hamilton syringe using stereotaxic equipment adapted to rats. The volume of ic injections was 10 µl for CRF, Ucn 1, and Ucn 2, and 5 µl for astressin$_2$-B.

Intracerebroventricular injections (icv) were performed through a 50 µl Hamilton syringe in conscious, pre-handled rats equipped with a chronic icv cannula, that was implanted under anesthesia with a mixture of ketamine hydrochloride (75 mg/kg) and xylazine (5 mg/kg) 10 days before the experiments. A 22 ga plastic guide cannula was implanted into the right lateral brain ventricle (coordinates: antero-posterior: –0.8 mm; lateral: –1.5 mm; dorsoventral: –3.5mm from bregma) and maintained in place by dental cement. At the end of the experiments, the correct location of the cannula was verified by injecting 10 µl of 0.1% toluidine blue dye.

Intravenous injections (iv, 0.1 ml) were performed into the right jugular vein after a small (1 cm) skin incision under a short (2-5 min) isoflurane anesthesia. Wounds were closed with suture.

Intraperitoneal (ip, 0.3 ml) and **subcutaneous injections** (sc, 0. 1 ml) were done by mild hand restraint in conscious rats.

Subdiaphragmatic vagotomy was performed by circular seromuscular myotomy of the esophagus 2 cm proximal from the gastro-esophageal junction in fasted rats under ketamine hydrochloride (75 mg/kg, ip) and xylazine (25 mg/kg, ip) anesthesia 36 hours before the experiments. Sham vagotomy consisted of a median laparotomy and similar manipulation of the esophagus and stomach without the myotomy under anesthesia. Then rats were fed with a liquid diet for 24 h post surgery, then they were deprived of food, but had free access to tap water, for 12 h prior to the gastric emptying measurements.

Gastric emptying (GE) of a non-nutrient viscous meal was determined by the phenol red method. Continuously stirred 1.5% (w/v) methylcellulose containing phenol red (50 mg/100 ml) was given intragastrically through a stainless steel gavage tube (in 1.5 ml volume) to conscious rats. At 20 min after the administration of the solution, rats were euthanized by CO_2 inhalation. The stomach was removed and homogenized in 0.1 N NaOH solution. The supernatant of suspension was added to 20% trichloroacetic acid (w/v) and centrifuged. The absorbance of the samples was read at 560 nm by a spectrophotometer. The absorbance of the phenol red recovered from animals euthanized immediately after gavage of the liquid meal was taken as a standard (0% emptying). The percentage of emptying during the 20-min period was calculated with the following formula: % emptying = (1 − absorbance of test sample/absorbance of standard) × 100.

RESULTS

Ucn 2 and Ucn 1 injected ic inhibit gastric emptying in conscious rats.

Ucn 2, injected ic at 0.03, 0.1 and 1 μg, dose-dependently inhibits gastric emptying of a viscous non-caloric solution in conscious rats. At 0.1 μg, ic Ucn 2 significantly reduced gastric emptying to 37.8 ± 6.9% compared with 58.4 ± 3.8% in the ic saline-treated group, while Ucn 1 had no effect (56.3 ± 0.5%). At 1 μg, both Ucn 2 and Ucn 1 injected ic induced a similar inhibition of gastric transit (23.1 ± 8.6% and 21.6 ± 5.9%, respectively, $P < 0.05$ compared with ic saline).

Astressin$_2$-B injected ic prevents ic Ucn 2- and ic CRF-induced delayed gastric emptying.

Astressin$_2$-B, injected at 3 and 10 µg, completely prevented ic Ucn 2-induced inhibition of gastric emptying (51.6 ± 2.8% and 54.3 ± 7.2%, respectively, vs. 36.6 ± 6.2%, $P < 0.05$), while at 1 µg ic, astressin$_2$-B was ineffective (27.9 ± 4.5%). Similarly, astressin$_2$-B injected ic at 3 µg completely antagonized ic CRF (0.3 µg)-induced inhibition of gastric emptying (52.0 ± 4.6% vs. 18.9 ± 4.8% $P < 0.05$). Astressin$_2$-B (3 µg) injected ic alone did not influence basal gastric emptying of a non-nutrient viscous meal (51.3 ± 6.6%).

Vagotomy blocked ic CRF, but not ic Ucn 2 -induced inhibition of gastric emptying.

Ucn 2, injected ic at 0.1 µg, did not significantly delay gastric emptying in sham-vagotomized rats (29.0 ± 9.5% vs. 34.6 ± 3.8%, respectively), while there was a significant reduction in vagotomized rats (15.6 ± 3.2% vs. 33.4 ± 5.9%). At 1 µg, ic Ucn 2 significantly reduced gastric emptying in sham-group (16.9 ± 2.9% vs. 34.6 ± 3.8%), and the inhibitory effect was significantly enhanced in vagotomized (6.3 ± 3.0%) compared to sham (16.9 ± 2.9%) groups. In sham-vagotomized rats, ic injection of CRF (1 µg) significantly delayed gastric emptying (9.7 ± 9.7%) compared to vehicle (34.6 ± 3.8%). Subdiaphragmatic vagotomy completely prevented ic CRF-induced delayed gastric emptying (45.5 ± 8.4%). Subdiaphragmatic vagotomy did not influence basal gastric emptying of viscous solution (33.4 ± 5.9%) compared with sham-operated rats (34.6 ± 3.8%).

It is to note that sham and vagotomized ic vehicle groups, fed liquid diet for 24 h post surgery followed by a 12 h fast had a lower gastric emptying compared with other non operated ic vehicle groups and fasted for 16-18 h. The shorter fasting time period along with the surgery, known to influence gastric emptying may have contributed to the lower gastric emptying in operated rats.

Bretylium prevents ic and icv Ucn 2-, but not iv Ucn 2-, ic CRF- or ic Ucn 1-induced inhibition of gastric emptying.

Ucn 2, injected ic (0.1 µg) in rats under short anesthesia or icv (1 µg) in lightly restrained conscious rats, significantly delayed gastric emptying (27.1 ± 7.3% and 22.7 ± 5.7%, respectively) compared to groups injected with saline either ic (55.8 ± 6.8%) or icv (67.6 ± 13.5%). The inhibitory effect of both ic and icv Ucn 2 was completely prevented by ip bretylium tosylate (56.1 ± 4.4% and 57.0 ± 8.7%, respectively). In contrast, neither ic CRF (0.3 µg) nor ic Ucn 1 (1 µg) -induced delayed gastric emptying (25.3 ± 4.0% and 19.7 ± 5.7,

respectively) was altered by pre-treatment with ip bretylium tosylate (18.5 ± 4.9% and 11.9 ± 4.4%, respectively). In addition, iv Ucn 2-induced significant suppression of gastric emptying (9.0 ± 3.2%) was not modified by bretylium tosylate pretreatment (5.0 ± 3.5%). In ic, icv or ip saline treated groups, ip bretylium tosylate did not modify gastric emptying compared to ip vehicle.

Prazonine prevents ic Ucn 2-induced inhibition of gastric emptying while yohimbine and propanolol had no effect.

Pretreatment with the α_1-adrenergic receptor blocker, prazosin (1 mg/kg, ip), completely abolished the delayed gastric emptying induced by ic Ucn 2 compared with vehicle pretreated group (56.9 ± 9.3% vs. 29.2 ± 3.0%). By contrast the α_2-adrenergic receptor blocker, yohimbine (4 mg/kg, sc) and β-adrenergic receptor blocker, propranolol (1 mg/kg, ip), did not modify Ucn 2 (0.1 μg, ic)-induced delayed gastric emptying. Prazosin, yohimbine and propanonol injected alone with ic saline did not alter significantly the 20 min basal gastric emptying values.

The peripheral injection of selective CRF_1 receptor subtype antagonist, CP-154,526 partially prevented iv IL-1β-induced delayed GE.

In the control group, rats injected sc with the vehicle of CP-154,526 and iv with the vehicle (0.1 ml/rat) had 20 min GE values of 49.8±3.5%. The iv injection of IL-1β in sc vehicle pretreated rats, reduced the GE to 9.6±2.6% ($P < 0.05$ vs control group). CP-154,526 (20 mg/kg) injected sc 30 min prior to the iv injection of IL-1β (500 ng/rat) partially prevented the delay in GE induced by IL-1β (34.8±9.8%, * $P < 0.05$ compared to CP 154,526 + iv vehicle and # $P< 0.05$ compared to sc vehicle + iv IL-1β treated group). CP-154,526 pretreatment did not influence basal GE (59.8±8.6%).

Central injection of the selective CRF_1 receptor subtype antagonist, CP-154,526 abolished iv IL-1β-induced delayed GE.

In the control group, rats injected with icv vehicle followed by iv vehicle, the 20-min-GE was 56.3 ± 1.1%. In rats petreated icv with vehicle, IL-1β (500 ng/rat, iv) inhibited GE to 24.3 ± 0.9% ($P < 0.05$ vs. control). Pretreatment with CP-154,526 (1 mg/rat, icv) completely antagonized the effect of IL-1β-induced delayed GE (55.6 ± 7.3% $P < 0.05$ vs icv vehicle + iv IL-1β treated group). CP-154,526 icv alone did not affect basal GE (57.8 ± 2.8%).

Peripheral injection of the CRF_2 receptor subtype antagonist, astressin$_2$-B partially prevented iv IL-1β-induced delayed GE.

The control group injected iv with saline followed by iv vehicle had a GE of 58.8 ± 7.3% in 20 min. IL-1β (500 ng/rat) inhibited the 20 min GE of the phenol red test meal to 9.0 ± 9.0% ($P < 0.05$ vs. control group). Intravenous injection of astressin$_2$-B (100 μg/kg) prevented the effect of IL-1β on GE (42.8 ± 10.0%, $P < 0.05$ compared to vehicle iv+ iv IL-1β treated group). Astressin$_2$-B did not modify basal GE compared to the control group (54.5 ± 4.1%).

The central injection of selective, CRF_2 receptor subtype antagonist, astressin$_2$-B failed to reverse IL-1β-induced delayed GE.

The ic injection of saline followed by the iv injection of IL-1β (500 ng/rat) significantly inhibited GE to 16.3 ± 1.5% compared to control group that received ic saline followed by iv (60.8 ± 4.3%, $P < 0.05$). Intracisternal administration of astressin$_2$-B (10 μg/rat) did not prevent IL-1β–induced delayed GE (27.5 ± 5.7%). Astressin$_2$-B (10 μg/rat, ic) did not influence basal GE compared to the controls (63.5 ± 5.0%).

Statistical Analysis

All results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test was performed for comparison between groups. *P* values < 0.05 were considered statistically significant.

DISCUSSION

The present study demonstrates that the newly characterized **CRF_2 selective agonist, Ucn 2** injected ic, inhibits gastric emptying of a viscous non-caloric solution in a dose-dependent manner in conscious fasted rats. Ucn 2 injected ic is more potent than Ucn 1. We observed a similar delay in gastric emptying in response to Ucn 2 injected in conscious fasted rats through a chronically implanted icv cannula. The selective CRF_2 antagonist, astressin$_2$-B injected ic, completely prevented both ic Ucn 2- and ic CRF-induced delayed gastric emptying of a liquid non-nutrient meal. The delayed gastric emptying induced by ic injection of Ucn 2, and the prevention of ic Ucn 2, ic CRF and ic Ucn 1 inhibitory action by the selective CRF_2 antagonist provide direct pharmacological evidence that the CRF_2 receptor is involved in ic CRF and urocortins action. Before the identification of CRF_2 agonists and

antagonists, indirect evidence were indicative of a role of brain CRF_2 receptors based on the rank order of potency of peptides to inhibit gastric emptying, which was in line with their differential affinity to CRF_2 receptor (sauvagine>urotensin-I>CRF).

Convergent evidence from this study indicates that sympathetic pathways and α_1-adrenergic receptor are involved in the inhibition of gastric emptying induced by central injection of Ucn 2. First, ip injection of bretylium, an adrenergic neuronal blocking agent taken up selectively at peripheral adrenergic nerve terminals, and blocking transmitter release from sympathetic postganglionic nerve terminals abolished both ic and icv Ucn 2-induced decreased gastric emptying. Second, subdiaphragmatic vagotomy did not block, but significantly enhanced, the inhibitory action of Ucn 2 injected ic at 0.1 and 1 μg resulting in a 56% and 82% suppression of gastric emptying. The increased inhibitory effect of ic Ucn 2 in vagotomized rats may be consistent with the sympathetic mediated inhibitory mechanisms that are no longer restrained by vagal cholinergic tone. The main neurotransmitters/neuromodulators in postganglionic sympathetic nerves are noradrenaline, ATP and neuropeptide Y. In the present study, pharmacological blockade of α_1-adrenergic receptors by prazosin mimicked the effects of bretylium while α_2 and β-adrenergic blockade by yohimbine and propanolol had no effect. Such a reversal of ic Ucn 2 action occurs under conditions where sympathetic or adrenergic blockade did not alter basal gastric emptying as previously reported for gastric emptying and motility in fasted and fed state rats. Based on these and other EMG studies gastric relaxation and α_1 receptor-mediated inhibition of phasic gastric contraction may play a role in the sympathetic α-adrenergic mediated delayed gastric emptying induced by ic Ucn 2.

Ucn 2 injected iv significantly suppressed gastric emptying of a liquid non-nutrient meal that action was not altered by bretylium pretreatment under conditions blocking ic or icv Ucn 2-induced delayed gastric emptying. These results established that the peripheral inhibitory action of Ucn 2 is mediated through distinct mechanisms from those elicited by central administration. Based on neuroanatomical and neurofunctional studies, it is likely that ic and icv Ucn 2 act at nucleus tractus solitarius (NTS), area postrema, paraventricular nucleus of the hypothalamus (PVN), central amygdala and dorsal motor nucleus (DMN) to influence sympathetic outflow and inhibit gastric motor function.

The present studies –based on results of experiments with bretylium and subdiaphtagmatic vagotomy- also provide evidence that ic CRF and Ucn 1 inhibit gastric emptying of a non-nutrient meal through distinct neural pathways than Ucn 2.

Although CRF displays high affinity to CRF_1 and a 40-fold lower affinity to CRF_2 receptors, and Ucn 1 has a high affinity to both CRF receptor subtypes, the selective CRF_2 antagonist, astressin$_2$-B, antagonized ic CRF- and Ucn 2-induced inhibition of gastric emptying of a liquid meal (present study) and ic Ucn 1-induced inhibition of a solid nutrient meal. These data support that ic CRF and Ucn 1 interact with CRF_2 receptors to induce a vagally-mediated inhibition of gastric motor function. However it cannot be discounted that astressin$_2$-B reversible effect on CRF/Ucn 1 may reflect co-activation of CRF_1 and CRF_2 receptors or initial activation of CRF_1 pathways that recruits brain medullary CRF_2 receptors.

The underlying mechanisms whereby preferential vagal vs. sympathetic pathways are recruited through activation of brain CRF_2 receptors by different members of the CRF family are still to be defined. Ucn 2 and CRF/Ucn 1 display differential chemical properties (selective affinity to CRF_2 receptors and low affinity to the CRF binding protein) that may play a role. The possibility of a third CRF receptor that is recognized by astressin$_2$-B and mediates the differential mechanisms of action of CRF/Ucn 1 and Ucn 2 cannot be ruled out.

Brain Ucn 2 may play a role in stress-related alterations of gastric motor function. First, we observed a potent action of ic Ucn 2 to suppress gastric emptying. Second, there is a similar central CRF_2 receptor-mediated sympathetic inhibition of gastric emptying induced by icv or ic Ucn 2 (present study) and restraint stress. Lastly, recent reports showed the presence of Ucn 2 mRNA in stress-responsive hypothalamic and brainstem nuclei, along with dramatic induction of Ucn 2 mRNA in the parvocellular part of the PVN by restraint.

IL-1β injected intravenously delays gastric emptying of a non-nutrient, semi-liquid test meal in fasted rats as previously described. In the present study, we showed that CP-154,526, a selective non-peptide CRF_1 antagonist, prevents the iv IL-1β-induced delayed gastric emptying completely when injected centrally (1 mg/rat, icv) and partially when injected peripherally (20 mg/kg, sc). Our results show more potent inhibitory action of CP-154,526, that crosses the blood-brain barrier, by central administration suggesting that the site of action is in the brain.

CRF_1 receptors located in CRF producing neurons of the parvocellular paraventricular nucleus of the hypothalamus are rapidly up-regulated reaching the peak expression level between 30 min to several hours following stress, depending on type of the stressor. CRF fails to altere basal gastric motility in non-stress situation, while it mimics the inhibitory action of several stressors. There is evidence also about immune stress (IL-1β) -induced activation of

CRF containing neurons in the hypothalamus. Taking these results and evidences together, we suggest that CP-154,526 action to inhibit IL-1β effect on gastric emptying is mediated through central CRF_1 receptors in the hypothalamus.

The fact that both urocortin ligands and CRF_2 receptors are expressed in the gastrointestinal tract supports local action of Ucn's in the modulation of gastrointestinal functions. Peripheral astressin$_2$-B (100 µg/kg) administered at a dose causing entire reversal of iv urocortin 2, CRF or restraint-stress, prevented the delay in gastric emptying induced by peripheral IL-1β in this study. However central administration of the selective peptide CRF_2 antagonist astressin$_2$-B (10 µg/rat) at a dose effective to reverse central CRF, Ucn 1 or Ucn 2 and restraint stress-induced inhibition of GE, failed to reverse the GE inhibitory action of iv IL-1β. We recently reported that CRF_1 deficient mice or wild type mice pretreated with a selective CRF_1 antagonist do not develop gastric ileus after abdominal surgery with cecal palpation, which data are consistent with findings of the present study performed on rats. The exact mechanism needs further evaluation.

Part II. Role of interferon-alpha in regulation of gastric secretion.

INTRODUCTION

Gastric acid secretion in health and disease is regulated by **neural, hormonal, paracrine,** and **autocrine** pathways at peripheral and central nervous system levels. The neural regulation of gastric secretory function has two levels, a system localized into the gastrointestinal organs (**intrinsic regulation**) and a central nervous system based **extrincic neurohumoral regulation**. Also the biologically active products of intraluminal **bacterium flora** and the **immune cells** localized in the gastrointestinal tract are able to interfere or modulate gastric secretion.

Central nervous system has a key function in **extrinsic regulation** of gastric secretory functions. The afferent fibers come from the receptors in the stomach wall and have synapses in the sympathetic paravertebral ganglia where the neurons are situated. The dorsal motor nucleus of the vagus nerve in the medulla and the paraventricular nucleus in the hypothalamus play crucial role in the integration of afferent and efferent information. The efferent fibers are located in the sympathetic and parasympathetic plexes and have synapses in the stomach wall or in the intramural myenteric or submucous plexus. Efferent ganglionic cells receive mainly

cholinergic stimulatory, in less ratio preganglionic parasympathetic inhibitory fibers through the vagus nerve, and inhibitory postganglionic fibers from the sympathetic plexes.

Nitric oxide (NO), a free radical is known to be a key mediator of the regulation of both gastric motility, secretion and mucosal blood flow. NO is a gas synthesized from L-arginine by way of the catalytic action of nitric oxide synthase (NOS). Enzyme NOS has constitutive (eNOS, nNOS) and inducible isoforms (iNOS) depending whether their NO production is continuous or can be induced by *a stimulus*. NO may function as a neurotransmitter, intracellular signal messenger, or paracrine agent. NO inhibits gastric acid secretion stimulated by several factors at the parietal cell level, both in vitro and in vivo. NO does not directly influence acid secretion in vivo but could play an **inhibitory modulator role** in neuronally mediated acid responses.

Different stressors (mild hyperthermia, intracisternally administered oxytocine or intravenous endotoxin) -induced stimulation of gastric acid secretion was blocked by microinjection of L-NAME into the dorsal motor nucleus of the vagus nerve (DMV) but not by injection to the nucleus of the solitary tract (NTS) in rats. Interleukin-1β, a key cytokine mediating immune responses to immunological challenges, and endotoxin-induced inhibition of pentagastrin-stimulated gastric acid secretion involves synthesis of NO from L-arginine.

Otherwise NO produced by eNOS in cells in close contact with parietal cells in human glandular gastric mucosa has been shown to have important role in inhibition of GAS stimulated by histamine or cAMP in vitro. Neuronal NOS (nNOS) has been also found in rat parietal cells in vitro.

Several reports established that CRF injected into the cerebrospinal fluid (CSF) either into the lateral brain ventricle (icv) or cisterna magna (ic) at nanomole doses induced a dose-related rapid onset and sustained inhibition of gastric acid secretion in conscious or anesthetized rats. Central CRF inhibits vagally stimulated acid secretion induced by either central injection of thyrotropin releasing hormone (TRH), 2-deoxy-D glucose, pylorus ligation, or gastric distention as well as the acid response to pentagastrin, while not altering that of histamine. The **CRF receptor subtype involved in CRF action needs to be further ascertained using selective CRF_2 agonists and antagonists.** Brain responsive sites have been identified in the **hypothalamus**, namely the VMH, PVN and LH nuclei in which microinjection of CRF induced a dose-related suppression of acid secretion in conscious pylorus-ligated rats. The lack of change in basal acid secretion in rats injected into the CSF with the CRF receptor antagonist indicates that **brain CRF receptors are not involved in basal acid secretion regulation, but play a role under stress-related conditions**.

The peripheral pathways through which central CRF inhibits gastric acid secretion involve the **autonomic nervous system,** gastric vagal efferent pathways and stimulation of sympathetic outflow.

In addition to stress, activation of central CRF signaling pathways is part of the brain mechanisms through which some anorexigenic brain peptides inhibit gastric acid secretion. Central injection of **interleukin-1β** in pylorus-ligated rats or peripheral administration of endotoxin-induced inhibition of stimulated acid secretion by gastric distention in urethane anesthetized rats are also not altered by ic injection of α-helical CRF_{9-41}.

Interferons (IFN's), a 165-172 amino acid cytokine family have key role in immune response to viral and parasitic infections and certain tumors. There are two main groups of interferons: type I interferons (IFN-α, IFN-β, IFN-ϖ) and type II interferon (IFN-γ). The production of **IFN-α** by virally infected cells induces resistance to viral replication, enhances MHC class I expression, increases antigen presentation, and activates natural killer cells to kill virus-infected cells. Members of the IFN`s family can be detected almost all type of tissues, i.e. in brain tissues, even in the absence of a specific inducer. Several biological stimuli, such viral, bacterial, mycoplasma or protozoa infections, exposure to certain cytokines and growth factors such interleukin-1, interleukin-2 or tumor necrosis factor highly increase IFN-α biosynthesis in CNS. It has been shown that treatment with IFN-α inhibited gastric emptying of isotope labelled solid food in humans, that could be reversed by cimetidine, a cholinergic and serotoninergic agonist. However there is little known regarding the effect of IFN-α on gastric secretion.

THE AIMS OF THESE SETS OF EXPERIMENTAL STUDIES:

1. to characterize the action of IFN-α on gastric secretion volume (GSV) and gastric acid secretion (GAS) in pylorus ligated rat model in two hours, and to determine the ED_{50} dose;
2. to determine whether the site of IFN-α action on inhibition of GSV and GAS induced by pylorus ligation is peripheral or is in the central nervous system in rats;
3. to determine the role of endogenous nitric oxide in mediation of central (ic) IFN-α–induced inhibition of GSV and GAS in rats;
4. to determine, whether production of NO by central or peripheral NOS is involved in mediation of centrally (ic) administered IFN-α induced inhibition of GSV and GAS in rats;
5. to determine whether the continuous or the inducible isoform of NOS is involved in the mediation of central (ic) IFN-α-induced inhibition of GSV and GAS induced by pylorus ligation in rats by using N^G-nitro-L-arginine methyl ester (L-NAME), a specific and non-selective competitive antagonist of the NOS isoenzymes and aminoguanidine, a selective inhibitor of inducible isoform of NOS.
6. to investigate the role of α and β adrenergic sympathetic pathways in mediation of central (ic) IFN-α-induced inhibition of GSV and GAS in pylorus ligated rats;
7. to investigate the role of endogenous prostaglandine pathways in mediation of central (ic) IFN-α-induced inhibition of GSV and GAS in pylorus ligated rats;
8. to investigate the role of central CRF_2 receptors in mediation of central (ic) IFN-α-induced inhibition of GSV and GAS in pylorus ligated rats;
9. to investigate the role of central CRF_1 receptors in mediation of central (ic) IFN-α-induced inhibition of GSV and GAS in pylorus ligated rats.

MATERIALS AND METHODS

Animals

Male CFY rats weighting 180-220 g were maintained under controlled housing conditions. Food (Standard Purina rat chow) and water were available ad libitum. Rats were deprived of food for 18 hours before the experiments, but had free access to water up to two hours before start of experiments. All experiments were performed between 10 a.m. and 2 p.m.

Drugs and substances

Human leucocyte interferon-α (**IFN-α**) was diluted in 0.9 % saline for ic and sc injection. **Nω-nitro-L-arginine methyl ester** (**L-NAME**), a competitive antagonist of constitutive and inducible isoforms of nitric oxide synthase enzyme (NOS), was dissolved in saline and injected iv (0.1 ml) or ic (5 μl) right before IFN-α or vehicle administration. **L-arginine**, the physiological substrate of NOS, and its D-stereoisomere, **D-arginine**, that is not substrate of NOS and L-NAME were dissolved in saline. Because of pharmacokinetical reasons to provide needed continuous serum level of the short-time turnover substrates, we administered L-arginine and D-arginine both sc (500 mg/kg) and iv (500 mg/kg) 5 minutes before injection of L-NAME (3 mg/kg, iv) and IFN-α or their vehicles. **Aminoguanidine-hidrogen carbonate**, a selective inhibitor of the inducible isoform of NOS (iNOS), was dissolved in saline (up to 0.5 ml) then injected ip (100 mg/kg) 60 minutes before ic IFN-α or 10 μl saline administration. **Indomethacin**, a cyclooxigenase enzyme type 1 and type 2 inhibitor was dissolved in 1% sodium bicarbonate and injected intraperitoneally (ip, 5 mg/kg) 60 minutes before ic injection of IFN-α or vechicle. **Phentolamine mesilicate**, a sympathetic alpha-1 and alpha-2 receptor blocker, and **propranolol chloride**, a sympathetic beta-1 and beta-2 receptor blocker, were solved in sterile saline right before experiments. **Anti-Sauvagine 30**, a selective CRF_2 receptor antagonist peptide, a**lpha-helical $CRF_{9\text{-}41}$**, a dominantly CRF_1 receptor, but also partially CRF_2 receptor antagonist peptide were dissolved in sterile saline right before the experiments. Inhalation of **diethyl-ether** was used for anesthesia during procedures.

Measurement of gastric acid secretion (GAS)

Animals were anesthetized with inhalation of diethyl-ether for 10 minutes (same time was applied for different injection route protocols), meanwhile pylorus was approached and ligated with width surgical thread. The muscular abdominal wall was closed by suture and the skin was clumped. Rats were euthanized by inhalation of carbon dioxide two hours later. Stomachs were then removed and their content was collected. Samples were centrifuged and the volume of supernatant (gastric secretion volume, **GSV**) measured and the acid content (**GAS**) was determined by titration to pH 7.0 with 0.1 N NaOH.

RESULTS

Results are expressed as means ± S.E.

Peripheral IFN-α inhibits gastric secretion volume (GSV) and gastric acid secretion (GAS) on a dose-dependent manner.

Subcutaneously administered IFN-α (1000, 10.000, 100.000 IU/rat) induced a dose-related inhibition of GSV (3.9±0.4 ml/2 hrs, 3.0±0.3 ml/2hrs, *2.4±0.2 ml/2hrs, respectively) and GAS (370±40 μmol/2 hrs, *233±39 μmol/2 hrs, *208±50 μmol/2 hrs, respectively) compared to subcutaneous saline treated control group (4.1±0.5 ml/2 hrs and 415±59 μmol/2 hrs, respectively) in pylorus ligated rats. * $P < 0.05$ compared to control group.

Central IFN-α inhibits GSV and GAS on a dose-dependent manner.

Intracisternally injected IFN-α (10, 100, 1000 IU/ rat) induced a dose-related inhibition of GSV (3.9±0.3 ml/2 hrs, *2.5±0.4 ml/2 hrs, *2.0±0.2 ml/2 hrs, respectively, Fig.12A) and GAS (481±50 μmol/2 hrs, *249±75 μmol/2 hrs, *141±25 μmol/2 hrs, respectively) compared to intracisternal saline treated control group (4.1±0.5 ml/2 hrs and 485±65 μmol/2 hrs) in pylorus ligated rats. From these results **100 IU IFN-α was found intracisternal ED_{50} dose**, which dose was used in the following experimens. * $P < 0.05$ compared to control.

Central IFN-α inhibits GSV and GAS more potently than peripherally suggesting its primary central site of action.

The central ED_{50} dose of IFN-α injected intracisternally induced inhibition of GSV (*2.5 ± 0.4 ml/2 hrs) and GAS (*177 ± 26 μmol/2 hrs) compared to control groups (4.0 ± 0.5 ml/2 hrs and 535 ± 37 μmol/2 hrs), respectively. However the intravenous injection of the same dose of IFN-α to the same weighting rats failed to induce an inhibition of GSV (4.3 ± 0.4 ml/2 hrs) or GAS (521 ± 38 μmol/2 hrs) compared to respective control animals (4.5 ± 0.5 ml/2 hrs or 497 ± 43 μmol/2 hrs). * $P < 0.05$ compared to control.

Central IFN-α–induced inhibition of GAS is mediated through NO pathways.

Intracisternal IFN-α (100 IU/rat) induced inhibition of GSV (*2.3 ± 0.4 ml/2 hrs compared to saline treated control group 4.0 ± 0.5 ml/2 hrs) and GAS (*280 ± 85 μmol/2 hrs versus controls 437 ± 66 μmol/2 hrs) was prevented by pre-treatment with intravenous 3 mg/kg L-NAME (#3.9 ± 0.5 ml/2 hrs and #507 ± 75 μmol/2 hrs, respectively). Controls were injected with vehicles.

L-NAME did not prevent IFN-α-induced inhibition of GSV or GAS in rats pre-treated with 500mg/kg iv and 500 mg/kg sc L-arginine (*2.1 ± 0.3 ml and *266 ± 82 μmol/2 hrs, respectively). L-arginine did not influence GSV or GAS in vehicle treated (4.3 ± 0.3 ml/2 hrs and 568 ± 59 μmol/2 hrs, respectively) or IFN-α treated animals (*2.7 ± 0.3 ml/2 hrs and *287 ± 48 μmol/2 hrs, respectively).

D-arginine (500mg iv and 500 mg/kg sc) that is no substrate of NOS failed to reverse L-NAME effect on IFN-α-induced inhibition of GSV (#4.9 ± 0.4 ml/2 hrs) and GAS (#630 ± 60 μmol/2 hrs). D-arginine did not influence GSV or GAS in vehicle treated (4.3 ± 0.4 ml/2 hrs and 550 ± 90 μmol/2 hrs, respectively) or IFN-α treated animals (*2.3 ± 0.2 ml/2 hrs, *230 ± 30 μmol/2 hrs, respectively). * $P < 0.05$ compared to vehicle control, # $P < 0.05$ compared to IFN-α treated group.

Peripheral L-NAME prevents IFN-α-induced inhibition of GSV and GAS more potently than through central route.

Intracisternal 100 IU IFN-α-induced inhibition of GSV (*2.3 ± 0.3 ml/2 hrs compared to 4.6 ± 0.5 ml/2 hrs of control group) and GAS (*250 ± 40 μmol/2hrs compared to 510 ± 50 μmol/2hrs of control group) was entirely prevented by 0.3 μg/kg and 3 μg/kg intravenous pre-treatment with L-NAME (#4.4 ± 0.4 ml/2 hrs, #4.5 ± 0.3 ml/2 hrs and #560 ± 60 μmol/2 hrs, #580 ± μmol/2hrs, respectively), however intracisternally injected 0.075 μg/rat (equals to 0.3 μg/kg dose) L-NAME failed to prevent IFN-α (100 IU, ic) inhibitory action (*2.5 ± 0.3 ml/2 hrs and *250 ± 40 μmol/2 hrs, respectively). Only 0.75 μg/rat (equals to 3 μg/kg dose) intracisternally administered L-NAME blocked IFN-α-induced inhibition of GSV (#4.6 ± 0.4 ml/2 hrs) and GAS (#550 ± 50 μmol/2hrs). L-NAME alone neither peripherally (0,3 μg/kg or 3 μg/kg) nor centrally (0.075 μg/rat or 0.75 μg/rat) did change GSV (4.5 ± 0.4 ml/2 hrs, 4.6 ± 0.5 ml/2 hrs or 4.6 ± 0.4 ml/2 hrs, 4.7 ± 0.4 ml/2 hrs, respectively) or GAS (530 ± 30 μmol/2 hrs, 590 ± 80 μmol/2 hrs or 570 ± 60 μmol/2 hrs, 570 ± 80 μmol/2 hrs, respectively). * $P < 0.05$ compared to vehicle control, # $P < 0.05$ compared to IFN-α treated group.

Inhibition of iNOS has no effect on IFN-α-induced inhibition of GVS and GAS.

Pre-treatment with subcutaneous aminoguanidine (100 mg/kg) failed to prevent IFN-α–induced inhibition of GSV or GAS (*2.5 ± 0.3 ml/2 hrs and *198 ± 40 μmol/2 hrs vs *1.4 ± 0.7 ml/2 hrs, *166 ± 20 μmol/2 hrs, respectively) in pylorus ligated rats. Aminoguanidine (100 mg/kg, sc) did not change significantly GSV and GAS (3.7 ± 0.4 ml/2 hrs and 377 ± 48

μmol/2 hrs) compared to vehicle controls (4.1 ± 0.3 ml/2 hrs, 452 ± 46 μmol/2 hrs). * $P < 0.05$ compared to vehicle treated control group.

Sympathetic α adrenergic pathways have key role in mediation of central IFN-α-induced inhibition of GSV and GAS.

The intracisternal injection of 100 IU IFN-α-induced inhibition of GSV (*2.5 ± 0.4 ml/2 hrs, compared to vehicle treated controls 3.7 ± 0.6 ml/2 hrs) and GAS (*249 ± 75 μmol/2 hrs compared to vehicle treated controls 434 ± 85 μmol/2 hrs) was prevented by subcutaneous administration of phentolamine (1 mg/kg) (#3.9 ± 0.3 ml/2 hrs and #448 ± 48 μmol/2 hrs, respectively). Animals treated with subcutaneous phentolamine and intracisternal saline (10 μl) did not change GSV (4.2 ± 0.4 ml/2 hrs) or GAS (486 ± 57 μmol/2 hrs) compared to the control group. * $P < 0.05$ compared to vehicle control, # $P < 0.05$ compared to IFN-α treated group.

Sympathetic β adrenergic pathways do not play a role in mediation of central IFN-α-induced inhibition of GSV and GAS.

The intracisternal injection of 100 IU IFN-α-induced inhibition of GSV (*2.5 ± 0.4 ml/2 hrs, compared to vehicle treated controls 4.1 ± 0.4 ml/2 hrs) and GAS (*241 ± 48 μmol/2 hrs compared to vehicle treated controls 498 ± 60 μmol/2 hrs) was not prevented by subcutaneous administration of propranolol (1 mg/kg) (*2.7 ± 0.3 ml/2 hrs and *261 ± 54 μmol/2 hrs, respectively). Animals treated with subcutaneous propranolol and intracisternal saline (10 μl) did not change GSV (4.7 ± 0.4 ml/2 hrs) or GAS (552 ± 78 μmol/2 hrs) compared to the control group. * $P < 0.05$ compared to vehicle control, # $P < 0.05$ compared to IFN-α treated group.

Prostaglandines do not play a role in mediation of central IFN-α-induced inhibition of GSV and GAS.

The intracisternal injection of 100 IU IFN-α-induced inhibition of GSV (*2.5 ± 0.4 ml/2 hrs, compared to vehicle treated controls: 4.0 ± 0.5 ml/2 hrs) and GAS (*177 ± 26 μmol/2 hrs compared to vehicle treated controls: 534 ± 37 μmol/2 hrs) was not prevented by subcutaneous administration of indomethacin (500 μg/kg) (*1.5 ± 0.3 ml/2 hrs and *191 ± 67 μmol/2 hrs, respectively). Animals treated with subcutaneous indomethacin (500 μg/kg) and intracisternal saline (10 μl) did not change GSV (4.6 ± 0.5 ml/2 hrs) or GAS (467 ± 56

μmol/2 hrs) compared to the control group. * P < 0.05 compared to vehicle control, # P < 0.05 compared to IFN-α treated group.

Central CRF_2 receptors play crutial role in mediation of central IFN-α-induced inhibition of GSV and GAS.

The intracisternally administered 100 IU IFN-α induced inhibition of GSV (*2.3 ± 0.4 ml/2 hrs) and GAS (*227 ± 35 μmol/2 hrs) compared to intracisternal saline treated controls (4.6 ± 0.5 ml/2 hrs and 578 ± 67 μmol/2 hrs). The inhibitory action of intracisternal IFN-α on GSV and GAS could be entirely prevented by intracisternal pre-treatment with 20 μg α-helical $CRF_{9\text{-}41}$ (4.8 ± 0.8 ml/2 hrs and 568 ± 127 μmol/2 hrs) or 10 μg antisauvagine-30 (3.8 ± 0.4 ml/2 hrs and 474 ± 50 μmol/2 hrs). Neither α-helical $CRF_{9\text{-}41}$ nor antisauvagine-30 did change GSV (4.6 ± 0.5 ml/2 hrs and 4.0 ± 0.6 ml/2 hrs, respectively) or GAS (549 ± 65 μmol/2 hrs and 460 ± 80 μmol/2 hrs, respectively). * P < 0.05 compared to vehicle control, # P < 0.05 compared to IFN-α treated group.

CRF1 receptors are not involved in mediation of central IFN-α-induced inhibition of GSV and GAS.

The intracisternal injection of 100 IU IFN-α-induced inhibition of GSV (*2.5 ± 0.4 ml, compared to vehicle treated controls: 4.0 ± 0.5 ml) and GAS (*177 ± 26 μmol/2 hrs compared to vehicle treated controls: 534 ± 37 μmol/2 hrs) was not prevented by intracisternal administration of CP-154,526 (1 mg) (*1.5 ± 0.3 ml and *191 ± 67 μmol/2 hrs, respectively). Animals treated with intracisternal CP-154-526 (1 mg) and intracisternal saline (10 μl) did not change GSV (4.6 ± 0.5 ml) or GAS (467 ± 56 μmol/2 hrs) compared to the control group. * P < 0.05 compared to vehicle control, # P < 0.05 compared to IFN-α treated group.

DISCUSSION

The present study characterized the inhibitory action of IFN-α on gastric acid secretion stimulated by pylorus ligation, a method acting through vago-vagal reflex and adrenoceptor stimulation. IFN-α injected both peripherally (sc) and centrally (ic) inhibited gastric secretion volume and GAS on a dose-related manner in pylorus ligated rats. 100 times lower dose central injections of IFN-α induced same level GAS as peripheral injections, suggesting that the primary site of action is in the CNS.

Intravenous administration of L-NAME, a specific and non-selective enzyme NOS inhibitor blocked central IFN-α-induced inhibition of GAS suggesting that IFN-α action is mediated through a nitric oxide (NO) pathway. L-arginine pre-treatment prevented L-NAME inhibitory effect on IFN-α induced inhibition of GAS as a proof that NO actually produced by NOS has a key role in the process. However pre-treatment with aminoguanidine, a selective iNOS inhibitor, failed to block this effect of IFN-α. Hypothalamus and dorsal motor nucleus of vagal nerve are suggested as affected regions in the process after their key role in NO-mediated central regulation of GAS and high interferon receptor concentration. However there are further investigations needed in this field in the future.

Results of studies included in the present thesis provided evidence also about the key role of sympathetic outwlow and the CRF system, specifically central CRF_2 (but not CRF_1) receptrors in mediation of IFN-α-induced inhibition of gastric acid secretion.

α1 and α2 adrenergic receptor blocker phentolamine, but not β1 and β2 adrenergic blocker propranolol pretreatment prevented IFN-α inhibitory action on GAS and GSV suggesting the important role of α adrenoceptor transmission. However the exact location of IFN-α receptor and the site of sympathetic receptors responsible for IFN-α effect mediation or modulation need to be determined in the future. Notwithstanding the lack of change of basal acid secretion by CRF and CRF mediated pathways further suggests that IFN-α does not play a role in regulation of basal gastric acid secretion, but plays a role under immunological challenge- (stress) realted conditions.

SUMMARY OF NOVEL RESULTS OF THE AUTHOR STATED IN THE PRESENT THESIS

1. Characterized the central dose-related inhibitory action of Ucn 2 on 20-minutes gastric emptying of a non-caloric viscous meal in conscious rats;

2. Provided direct evidence about the key role of central CRF_2 receptor pathway of intracisternal Ucn 2 and CRF-induced inhibition of gastric emptying of a non-caloric viscous meal in conscious rats, using the selective CRF_2 antagonist, astressin$_2$-B, and proved that intracisternal Ucn 2 inhibits GE more potently than Ucn 1 does;

3. Provided evidence about that intracisternal CRF_2 receptor selective agonist Ucn 2 inhibits GE through increasing sympathetic outflow and vagotomy potentiates its effect, however CRF_1/CRF_2 receptor binding agonists CRF and/or Ucn 1-induced GE inhibitory action is mediated through the vagus nerve, and ganglionic blocade does not impair it in rats. These results highlight the role of CRF_1 receptors not proven before;

4. Provided evidence about key role of central, and less potently peripheral CRF_1 receptors in mediation of interleukin-1β-induced inhibitory action of gastric emptying of a viscous, non-caloric meal in rats, using the selective CRF_1 antagonist, CP-154,526;

5. Provided evidence about key role of peripheral but not central CRF_2 receptors in mediation of peripherally administered interleukin-1β-induced inhibitory action of gastric emptying of a viscous, non-caloric meal in rats, using the selective CRF_2 antagonist, astressin$_2$-B;

6. Characterized the dose-related inhibitory action of peripheral and central IFN-α–induced inhibition of gastric secretion volume and gastric acid secretion induced by pylorus ligation in rats in a 2-hours lasting model;

7. Provided indirect evidences about 100 times more potent gastric secretion volume and gastric acid secretion inhibitory action of centrally (ic) administered IFN-α than when injected peripherally (sc or iv) in a pylorus ligated rat model, suggesting that the primer site of action is in the central nervous system;

8. Provided evidence about key role of nitric oxide (NO) produced by nitric oxide synthase (NOS) in mediation of centrally (ic) administered IFN-α -induced inhibition of gastric secretion volume and gastric acid secretion in rats;

9. By comparison of central (ic) and peripheral (iv) administration routes of L-NAME, a NOS inhibitor, suggested that the central (ic) IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion is mediated mainly through central NO pathways in rats;

10. By testing with inhibitor of inducible NOS aminoguanidine provided indirect evidence about key role of constitutive isoforms of NOS in the mediation of central (ic) IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion in rats;

11. Provided evidende about key role of sympathetic adrenergic α_1 receptors in mediation of central (ic) IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion in rats, and proved that adrenergic β_1, β_2 and α_2 receptors are not involved in the process;

12. Proved that prostaglandines are not involved in the mediation of IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion in rats;

13. Provided evidence about key role of central CRF2 receptors in mediation of central (ic) IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion in rats;

14. By using selective non-peptide CRF1 receptor antagonist CP-154,526 demonstrated that central CRF1 receptors are not involved in central (ic) IFN-α-induced inhibition of gastric secretion volume and gastric acid secretion in rats.

4. Acknowledgements

Hereby I would like to express my many thanks:

To **Prof. Dr. Gyula Mózsik,** my mentor, for his leadership of my program and scientific and clinical work and for his support in my personal career.

To **Dr. Gábor Sütő**, my tutor, for his leadership of my work, the support in my scientific and clinical work and my personal career.

To **Dr. Ágnes Király** for her support in my scientific and clinical work and my personal career.

To **Prof. Dr. Yvette Taché**, my mentor, in the United States, for her leadership in my scientific work and for her support of my career and hospitality at the University of California, Los Angeles (UCLA).

To **Dr. Million Mulugeta** for his tutorship in the United States, for his support in my scientific work and his hospitality at the UCLA.

To **Dr. Andrew Luckey**, my collague and co-worker at the UCLA, for his support in my scientific work.

To **Prof. Dr. Kálmán Tóth** and **Prof. Dr. Béla Hunyady** for their leadership and support of presenting and defending my Ph.D. thesis.

To **Dr. Imre Szabó jr., Dr. György Rumi jr., Dr. András Debreceni, Dr. Oszkár Karádi, Dr. Beáta Bódis, Dr. Zsuzsanna Nagy, Prof. Dr. Mária Figler, Dr. Ferenc Pakodi, Dr. Beáta Gasztonyi, Prof. Dr. Alajos Pár, Dr. Gabriella Pár, Prof. Dr. Tibor Past**, **Dr. Levente Szabó, Dr. Leventéné Szabó, Merklerné Beáta Meng, Ágnes Bodrog** for their support of my scientific and clinical work at the 1st Department of Medicine of University of Pecs.

To **Dr. Hong Yang, Dr. Lixin Wang, Dr. Paul Saunders, Dr. Hovsep Kosoyan, Dr. David Adelson** for their support of my scientific work at the University of California, Los Angeles.

To **Paul Kirsch** and **Theresa Olivas** for their kind help and assistance in preparation of manuscripts at the University of California, Los Angeles.

To **Jánosné Jermás, Dr. Lajosné Nagy, Emőke Czérna, Edit Szilasné Szalay** for their assistance and help in my work.

To ***my parents*** and my brother, ***Dr. Roland Czimmer,*** for their support in my life and work.

To my wife, ***Dr. Éva Király*** for her support in my life and work and for providing a secure background in our family.

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