Ph.D. Thesis

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The role of neuropeptides and cytokines in the brain regulation of gastric motor function

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Content

Abbreviations:

ACTH:	adrenocorticotrophin hormon
CGRP:	calcitonin gene-related peptide
CNS:	central nervous system
CRF:	corticotropin releasing factor
DMN:	dorsal motor nucleus of the vagus
h	hour
$hCGRP_{8-37}$:	human calcitonin gene-related peptide 8-37 amino acid fragment
HPA axis:	hypothalamic-pituitary-adrenal axis
i.c.:	intarcisternal(ly)
IL-1:	interleukin-1
IL-1ra:	interleukin-1 receptor antagonist
i.p.:	intraperitoneal(ly)
i.v.:	intravenous(ly)
Paf:	platelet activating factor
PBMCs	peripheral blood mononuclear cells
PG:	prostaglandin
PVN:	paraventricular nucleus of the hypothalamus
TNF:	tumor necrosis factor

1. Introduction.

The gastrointestinal motility is a well organized process to result in the most efficient utilization of different nutrients ingested. One of the first steps of this processing is the mixing of food with digestive excretions (saliva, gastric acid and enzymes) followed by the delivery of the chyme to the small bowel. The rate of the gastric emptying along with the pancreatic excretion and gall bladder contraction is well coordinated to provide the most efficient absorption within the proximal small intestine. In the past two decades a new line of evidence was established how neural and immune system is involved in the organization of the gastric motility/emptying. Recently many data were raised to find a role for the mediators released during the activation of the immune system as well. A complex multidirectional communication exists among the immune, endocrine and neural systems through hormonal, paracrine and neuronal signals to get in concert in the regulation of gastrointestinal motility in response physiological or pathophysiological stimuli.

1.1. The concept of stress and its implications on different gastrointestinal functions.

Tissue injury or infectious diseases activate the body's defense mechanisms to conserve physiologic homeostasis (Kushner, 1982). At the site of injury or infection a local inflammation develops which is accompanied by systemic changes. These latter events are designated as the acute phase response. The first event has the role to remove the injurious agents(s) and to facilitate the local repair mechanisms. The acute phase reaction

helps the living organism to survive the damage mobilizing the host's defense mechanisms. Hans Selye has described the concept of stress in 1936 (Selye, 1936). His original observation was that the living organism reacts with a specific reaction to different nonspecific stimuli causing stress such as physical damage (e.g. cold, heat, injury), chemical compounds (toxic injuries) and psychologic factors. The endocrine changes (e.g. activation of the hypophyseal-adrenocortical axis and the adrenal medulla) of this reaction are accompanied by specific morphologic changes: adrenal hyperplasia, lymphoid and thymic involution and gastric mucosal lesions (Selye, 1936; Szabo et al., 1990). One classic component of the triad of the symptoms developing in response to a stressor is the gastroduodenal ulceration. According to this observation attention has been focused on other gastroenteric disorders related to stress such as altered gastrointestinal motility. It is well established that after a stressful stimulus the gastric emptying is delayed or completely stopped while the colonic motility is increased. These motility alterations result in or are associated with different clinical symptoms such as abdominal fullness, diarrhoea or loss of appetite.

1.2. Control of the gastrointestinal motility during stress.

The notion that the central nervous system (CNS) influences gastrointestinal motor functions came from the observation that emotions and stress influence gastrointestinal motility and propulsion. The first report was taken by P.J.G. Cabanis nearly 200 years ago, negative emotions get the gastrointestinal system cease digestion (Cabanis, 1802). Walter Cannon has observed that

stress results in the alteration of gastric motor activity (Cannon, 1902). In spite of these early observations a long time must have been elapsed for the brain- gut interactions to get in focus of gastrointestinal research. It is evident that different neuropeptides act at different chemosensitive sites in the central nervous system or the periphery. The key mediator of stress induced gastrointestinal motility disturbances seems to be the corticotropin releasing factor (CRF).

Exogenous injection of CRF into specific brain sites and endogenous activation of brain CRF pathways by stress are well established for their role in altering gastrointestinal function (Taché et al., 1993). CRF differently influences the upper or lower gastrointestinal tract. In particular, CRF injected into the cerebrospinal fluid or PVN or activation of CRF neurons in the PVN by various stressors inhibits gastric emptying and stimulates colonic motor function through an action mediated by the autonomic nervous system (Taché et al., 1993). This is independent of the endocrine effect of the peptide (Taché et al., 1993).

Many locations of CRF also were established outside the CNS such as peripheral T and B lymphocytes (Stephanou et al., 1990), testes (Dufau et al., 1993), trophoblastic and decidual tissue (Petraglia et al., 1990), pancreas (Petrusz et al., 1983), different types of cancers (Suda et al., 1984a) and the human gastrointestinal tract (Kruseman et al., 1982; Kawahito et al., 1994), or capsaicin sensitive primary afferent neurons (Skofitsch et al., 1984; Kamilaris et al., 1992). CRF injected not only i.c. but i.v. delays gastric emptying (Taché et al., 1987; Sheldon et al., 1990; Barquist et al., 1992).

1.3. The role of Interleukin-1β in the acute phase reaction.

The term interleukin (IL) was first proposed in 1979 (Dinarello, 1984a). The name interleukin-2 was given to a compound originated from lymphocytes which stimulated T-cell proliferation. That time macrophage products bearing various properties of interleukin-1 had been known under different names. Now it is widely accepted that endogenous pyrogen, lymphocyte-activating factor, leukocytic endogenous mediator are collectively referred to as interleukin-1 (Dinarello, 1984a). IL-1 exists in two forms alpha and beta which are the products of distinct genes sharing only 26% of amino acid homology (Dinarello, 1988). However the two forms of IL-1 binds to the same receptor and exerts a similar biological activity the alpha form is less potent than the beta (Katsuura et al., 1985; Rivier et al., 1989b; Ishikawa et al., 1990; Saperas et al., 1990;). IL-1β is a very potent molecule acting in the femtomol range (Saperas et al., 1990; Uehara et al., 1990). There are two receptors for interleukin-1: the 80 kilodalton type I receptor was characterized on T cells/fibroblasts, the 60 kilodalton type II receptors are present on B cells and macrophages (Dinarello et al., 1991). The central nervous system action of IL-1β is mediated by type I receptors (Farrar et al., 1987). A physiologic antagonist compound of IL-1 (interleukin-1 receptor antagonist: IL-1ra) was isolated form IgG activated monocytes which is structurally very closely related to IL-1 (Carter et al., 1990; Eisenberg et al., 1990; Hannum et al., 1990; Arend, 1991; Dinarello et al., 1991). IL-1ra shows a lack of intrinsic activity (Dinarello et al., 1991), and in previous experiments a 1000 fold excess of IL-1ra is required to block different

actions of IL-1β (Hetier et al., 1988; Dinarello et al., 1991; Saperas et al., 1992a; Saperas et al., 1993).

1.4. Central nervous system action of IL-1β.

1.4.1. Localization of IL-1β and IL-1 receptors within the central nervous system.

IL-1β was originally described as the primary product of immune cells (Dinarello, 1984a; Oppenheim et al., 1986; Dinarello, 1988), but recently a neuromodulator/neurotransmitter role is being emphasized for this peptide (Rothwell, 1991): (1.) Immunoreactive IL-1β was found in the rat forebrain both in hypothalamic and extrahypothalamic sites involved in the regulation of hypophysiotropic, autonomic, limbic and extrapyramidal functions (Breder et al., 1988; Lechan et al., 1990). (2.) IL-1 is released mostly by microglial cells and, to a lesser extent, by astrocytes (Giulian et al., 1986; Nieto-Sampdero et al., 1987; Hetier et al., 1988; Fabry et al., 1993; Lee et al., 1993). (3.) In response to damage or convulsion an excess amount of IL-1β or IL-1mRNA is expressed (Nieto-Sampdero et al., 1987; Higgins et al., 1991; Minami et al., 1991) in the brain. (4.) High affinity IL-1β receptors are localized and expressed primarily in association with neurons in the brain (Farrar et al., 1987; Katsuura et al., 1988a; Lechan et al., 1990; Takao et al., 1990; Cunningham et al., 1993).

There are several implications how circulating interleukin-1 can reach brain to influence different functions (Hashimoto et al., 1991): (1.) IL-1 binds to endothelial cell IL-1 receptors resulting in the production of further messengers such as

arachidonate metabolites (Dejana et al., 1987), nitric oxide (Lin et al., 1996) or IL-1 itself (Warner et al., 1987). (2.) There is also evidence that, systemic IL-1β can reach the brain (Banks et al., 1989; Banks et al., 1991a; Hashimoto et al., 1991; Cunningham et al., 1992;) through a saturable, carrier mediated transport system (Banks et al., 1991b), (3.) Large molecules such as IL-1β may penetrate the blood-brain barrier at sites where it is fenestrated (organum vasculosum laminae terminalis) to reach CNS neurons, (4.) IL-1β may have a peripheral receptor in the liver to stimulate hepatic afferents (Niijima, 1992). The central action of IL-1β are long lasting since IL-1 degrades very slowly within the central nervous system (Banks et al., 1991a; Banks et al., 1991b) and the cytokine induces its own gene expression and production (; Walter et al., 1989; Wang et al., 1991).

1.4.2. Interaction between IL-1 and CRF.

There are strong evidence that IL-1 may interact with CRF containing neurons resulting in their activation: Peripheral or central injection of IL-1ß activates corticotrophin releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN) and the release and synthesis of hypothalamic CRF (Sapolsky et al., 1987; Bernardini et al., 1990a; Bernardini et al., 1990b; Saphier et al., 1990; Ju et al., 1991; Navarra et al., 1991; Watanabe et al., 1991). There is evidence that prostaglandins (PGs) may be part of the mechanisms mediating hypothalamic CRF release (Katsuura et al., 1985; Bernardini et al., 1990a; Bernardini et al., 1990b; Katsuura et al., 1990; Navarra et al., 1991).

1.4.3. Effects of IL-1β on different gastrointestinal functions.

Circulating IL-1β may reach the gastrointestinal system or neural sites which are in association with the regulation of different gastrointestinal functions. There are several lines of evidence which have shown that IL-1β is expressed within the gastrointestinal system during illness or damage: (1.) In patients suffering from inflammatory bowel disease a higher level of IL-1 was detected in intestinal biopsies (Brynskov et al., 1992; Reimund et al., 1996), (2.) Rat gastric fundus stripes produced measurable amount of IL-1β (Montuschi et al., 1996), (3.) IL-1β is the putative mediator of the changes of cholinergic nerve function since IL-1β suppressed the release of acetylcholine from isolated rat myenteric plexus (Main et al., 1993).

IL-1β administered peripherally (i.v. or i.p.) or centrally (i.c. or i.c.v. or into specific brain nuclei) alters different gastric functions such as gastric acid and pepsin secretion, gastric emptying, intestinal and colonic motility. IL-1β was also shown to protect the gastric mucosa against various damaging agents (Uehara et al., 1990; Wallace et al., 1990; Okumura et al., 1991; Robert et al., 1991a; Robert et al., 1991b; Shibasaki et al., 1991; Perretti et al., 1992; Uehara et al., 1992a; Wallace et al., 1992).

1.4.3.1. Effects of IL-1 on gastrointestinal secretion.

Human recombinant IL-1β injected into the cerebrospinal fluid (i.c. or i.c.v.) impairs gastric secretion of conscious

rats with pylorus ligation (Ishikawa et al., 1990; Saperas et al., 1990; Uehara et al., 1990). Mainly the volume of gastric acid secretion was smaller, the decrease of acid concentration contributed to a lesser extent to the reduction of gastric acid output (Ishikawa et al., 1990; Saperas et al., 1990; Uehara et al., 1990). IL-1β is more potent (Ed_{50}= 4.5 ng) and has a longer lasting effect than peptides such as bombesin, calcitonin, calcitonin gene related peptide, CRF, opioid peptides and neurotensin injected centrally to inhibit gastric acid secretion. This phenomenon may be related to the slow degradation of IL-1 within the central nervous system (Banks et al., 1991a; Banks et al., 1991b) or by the induction its own gene expression and production (Walter et al., 1989; Wang et al., 1991). Recombinant human IL-1β injected i.p. at various doses reversed the net water absorption of the proximal colon into secretion (Theodoru et al., 1994). This action of IL-1β was dose dependent, involved neural elements and mast cell degranulation with the release of mediators such as prostaglandins (Theodoru et al., 1994).

1.4.3.2. Effects of IL-1 on gastrointestinal motility.

Peripheral administration of IL-1ß either i.p. or i.v. is also known to inhibit gastric emptying of a solid meal or a methylcellulose solution (Robert et al., 1991a; Robert et al., 1991b; McCarthy et al., 1992; van Miert et al., 1992). However, the mechanisms through which peripheral administration of IL-1ß suppresses gastric emptying are still not known. Since peripheral injection of IL-1ß stimulates PVN neurons containing CRF as shown by c-fos expression in this nuclei (Chan et al., 1993; Brady et al., 1994; Ericsson et al., 1994) and results in CRF release

(Berkenbosch et al., 1987; Sapolsky et al., 1987; Barbanel et al., 1989; Tsagarakis et al., 1989; Saphier et al., 1990; Suda et al., 1990; Watanabe et al., 1990; Ju et al., 1991; Watanabe et al., 1991). Data have shown a role for prostaglandins (PGs) in the release of CRF by IL-1 (Bernardini et al., 1990a; Bernardini et al., 1990ba; Navarra et al., 1991). Based on these data, gastric stasis induced by peripheral IL-1ß may involve central prostaglandin mediated CRF release.

IL-1ß delivered into the cerebrospinal fluid (CSF) inhibits gastric emptying of a non nutrient semi-liquid meal while stimulating colonic motility through central CRF dependent mechanisms in rats (Fargeas et al., 1993).

1.5. The role of bacterial lipopolysaccharide in the regulation of different gastrointestinal pathological conditions.

1.5.1. Bacterial lipopolysaccharide.

Lipopolysaccharide (LPS), the major constituent of the outer layer of gram negative microorganisms, is the most well known molecule released from infecting bacteria which stimulates the immune system to defend against infection. The chemistry of LPS was primarily elucidated by Westphal: endotoxin consists of a core polysaccharide, the O-specific antigen and the lipid A which determines the biologic activity (Galanos et al., 1977; Westphal et al., 1983). LPS according to its local action to result in inflammation at the site of infection may enter the circulation resulting in profound systemic effects, such as fever, acute phase reaction, neutropenia or in the most severe cases septic

shock or disseminated intravascular coagulation (Cybulsky et al., 1988). Injection of LPS into experimental animals mimics the symptoms of gram negative bacterial infections or septic shock as well.

1.5.2. Effects of bacterial LPS on the hosts defense mechanisms.

Endotoxin injection results in the release of cytokines interleukin-1β (IL-1β) and tumor necrosis alpha (TNFα) (Sirko et al., 1989; Zuckerman et al., 1989; Ulich et al., 1990; Foster et al., 1993). According to this widely accepted macrophage originated mediators of LPS action, several other compounds were suggested to be involved in different actions of LPS: arachidonic acid metabolites (Sirko et al., 1989), platelet-activating factor (Paf) (Whittle et al., 1987), nitric oxide (Nava et al., 1992), calcitonin gene-related peptide (CGRP) (Griffin et al., 1992; Wang et al., 1992b; Hüttemeier et al., 1993), and opioid peptides (Hamilton et al., 1986; Ulich et al., 1990).

1.6. Interaction between LPS and the CNS.

Recent studies indicate that the action of endotoxin is mediated at least partly by the central nervous system (CNS). Endotoxin was found to activate the hypothalamic-pituitary-adrenal (HPA) axis (Rivier et al., 1989a; Long et al., 1990), to suppress plasma gonadotropin levels (Long et al., 1990; Rivier, 1990), to induce IL-1β within the CNS (Koenig et al., 1990; Hillhouse et al., 1993; Wan et al., 1993) and other peripheral sites (Ulich et al., 1990) as well. The pathway of the activation

of the HPA axis depends on the dose of LPS administered: (1.) low doses utilize macrophage produced IL-1β, (2.) high doses activate the HPA axis in a macrophage-independent manner, releasing IL-1, IL-6 or PGE_2 from endothelial cells (Derijk et al., 1991; Tilders et al., 1994). LPS activates the sympatoadrenal system, acting at dual sites: (1.) the plasma norepinephrine level is dependent on the CNS, (2.) whereas the plasma epinephrine response involves both central and peripheral regulation (Zou et al., 1993). LPS administration both into the central nervous system or to peripheral sites (i.v. or i.p.) produces c-fos in medullary and hypothalamic sites which are involved in the regulation of different autonomic functions (Wan et al., 1993; LePard et al., 1994). The vagus nerve is involved in the CNS-mediated response to LPS since (1.) subdiaphragmatic vagotomy suppresses the CRF and ACTH release stimulated by a low dose of LPS (Gaykema et al., 1995), (2.) hepatic vagotomy abolishes LPS or lithium chloride-induced hyperalgesia (Watkins et al., 1994).

1.7. The role of capsaicin-sensitive primary sensory afferents and CGRP in the regulation of gastric motility.

Capsaicin sensitive primary sensory afferents are involved in the postprandial regulation of the upper gastrointestinal tract (Holzer et al., 1994; Raybould et al., 1994; Zittel et al., 1994) and in ileus due to surgery and/or peritoneal irritation (Holzer et al., 1986; Holzer et al., 1992; Plourde et al., 1993b; Takeuchi et al., 1996) in rats. α-CGRP an alternative splicing product of calcitonin gene (Rosenfeld et al., 1983) is abundantly localized within the gastrointestinal tract. Mostly it is stored

in and released from primary sensory afferent fibres arising from the dorsal root ganglia, a smaller amount was shown in fibres originating from nodose ganglia (Sternini, 1992). CGRP injected intravenously (i.v.) potently inhibits gastric acid secretion, motility and the development of mucosal lesions (Maggi et al., 1987; Taché, 1991; Taché et al., 1992a). The C terminal fragment of human CGRP, $hCGRP_{8-37}$ is a potent antagonist of CGRP on type 1 receptors (Chiba et al., 1989; Dennis et al., 1990; Donoso et al., 1990). CGRP was shown to inhbit gastric emptying through type 1 receptors, since $hCGRP_{8-37}$ reversed the inhibiton of gastric emptying induced by the i.v. injection of CGRP (Plourde et al., 1993a). According to the neuronal localization of CGRP a non neural, source was also described: it is highly likely that gastric mucosal lymphocytes synthesize CGRP mRNA and CGRP as well (Jakab et al., 1993).

1.7.1. Interaction between calcitonin gene-related peptide and LPS.

It is well established that LPS results in a marked increase of plasma calcitonin gene-related peptide during endotoxic shock, and it seems to mediate hemodynamic changes during endotoxicosis (Wang et al., 1991; Griffin et al., 1992; Wang et al., 1992a; Wang et al., 1992b; Hüttemeier et al., 1993). Prostaglandins were found to trigger CGRP release during the development of endotoxicosis (Wang et al., 1992a), dexamethasone was found to attenuate the increase of plasma CGRP content after LPS challenge of experimental animals (Wang et al., 1991).

1.8. Effect of LPS on different gastrointestinal functions.

LPS injection into experimental animals alters different gastric and intestinal functions. LPS inhibits gastric acid secretion (Uehara et al., 1992b; Saperas et al., 1994; Terao et al., 1995), impairs gastric mucosal blood flow and mucosal integrity (Whittle et al., 1987; Pique et al., 1988;) and alters colonic water and electrolyte transport (Ciancio et al., 1992). LPS administration disrupts intestinal motility (Pons et al., 1989) and increases intragastric pressure in rats (Esplugues et al., 1989). Little is known about the mediators of the gastrointestinal effect of LPS. Platelet activating factor (Paf) and PGs are involved in intestinal motor alterations (Pons et al., 1989): Paf seems to mediate intragastric pressure (Esplugues et al., 1989) after LPS treatment.

1.9. Aims of the experiments.

(1.) To investigate the influence of intracisternal (i.c.) or intravenous (i.v.) injection of IL-1β on gastric emptying in conscious rats.

(2.) To assess the mechanisms whereby IL-1ß exerts its inhibitory effect using specific receptor antagonists and inhibitor of prostaglandin synthesis. A special attention was focused on:

(a.) IL-1 receptors,

(b.) prostaglandins,

(c.) peripheral and central CRF.

(3.) To investigate the mechanism of action of the inhibition of gastric emptying by i.p. LPS, a potent stimulant of the immune system.

(4.) The role of

(a.) IL-1β,

(b.) peripheral and central CRF,

(c.) capsaicin sensitive primary sensory afferents and calcitonin gene related peptide

was studied in details in the pathogenesis of delayed gastric emptying following LPS injection.

2. Interleukin-1ß Acts in the Brain to Inhibit Gastric Emptying in Rats: Mediation Through Prostaglandin and CRF[1].

2.1. Background.

Interleukin-1 (IL-1) is one of the key mediators involved in immunological and pathological responses to infection and antigenic challenges (Oppenheim et al., 1986; Dinarello, 1988). In addition to its immune effects, IL-1 has been implicated as a neurotransmitter/neuromodulator in the central nervous system (CNS) (Rothwell, 1991). IL-1 is produced in the brain by microglial cells and, to a lesser extent, by astrocytes (Giulian et al., 1986; Hetier et al., 1988; Lee et al., 1993) as well as microvessel endothelium (Fabry et al., 1993). IL-1β-like immunoreactivity is also found constitutively in hypothalamic neurons regulating neuro-endocrine functions (Breder et al., 1988; Lechan et al., 1990) and high affinity IL-1β receptors are localized and expressed primarily in association with neurons in the brain (Farrar et al., 1987; Katsuura et al., 1988a; Takao et al., 1990; Cunningham et al., 1992; Cunningham et al., 1993). There is also evidence that, systemic IL-1β can reach the brain through a saturable, carrier mediated transport system (Banks et al., 1989; Reimers et al., 1991; Banks et al., 1991a) and that IL-1β induces activation of CRF-containing neurons in the PVN of the hypothalamus (Ericsson et al., 1994, Ericsson et al., 1997).

IL-1ß administered into the cerebrospinal fluid or specific hypothalamic nuclei influences endocrine, thermoregulatory, behavioral and immune systems (Rothwell, 1991). In addition,

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recent studies indicate that central injection of IL-1β induces marked changes in gastrointestinal function inhibiting gastric acid secretion and erosions and postprandial intestinal motility while stimulating cecocolonic motor activity (Taché et al., 1992b; Fargeas et al., 1993). Central or peripheral injection of IL-1ß activates corticotropin releasing factor (CRF)- containing neurons in the hypothalamus and the release of CRF through prostaglandin E_2 pathways (Berkenbosch et al., 1987; Sapolsky et al., 1987; Barbanel et al., 1989; Bernardini et al., 1990a; Suda et al., 1990; Saphier et al., 1990; Ju et al., 1991; Navarra et al., 1991; Watanabe et al., 1991; Chover-Gonzalez et al., 1993). Such an activation of PGE_2 and CRF in the brain has been involved in mediating several CNS actions of IL-1ß (Rothwell, 1991; Taché et al., 1992b; Fargeas et al., 1993). Central injection of CRF or endogenous CRF released in the brain in response to stress is well established to inhibit gastric motor function through modulation of the autonomic nervous system (Taché et al., 1993). These observations suggest that IL-1ß can act in the brain to inhibit gastric motor function through release of these transmitters.

In the present study, we investigated the influence of intracisternal (i.c.) injection of IL-1β on gastric emptying in conscious rats. The mechanisms whereby IL-1ß exerts its inhibitory effect were also assessed, in particular, the mediation through IL-1 receptors, and the role of CRF using a recently developed novel specific receptor antagonists, $[DPhe^{12}, Nle^{21,38}, C^{\alpha}MeLeu^{37}]CRF_{12-41}$ (Hernandez et al., 1993; Howard et al., 1993) and indomethacin as an inhibitor of prostaglandin synthesis (Saperas et al., 1991).

2.2 Materials and Methods.

2.2.1. Animals.

Male Sprague-Dawley rats (Harlan Sprague Dawley, San Diego, CA) weighing 250-300 g, were maintained under controlled housing conditions of light (6 a.m. to 6 p.m.), humidity (35%-55%), and temperature (22 ± 2°C) for one week. Food and water were available ad libitum. Rats were deprived of food for 18 h before the experiments but had access to water up to the time where gastric emptying was measured. All the experiments were performed between 12 p.m. and 4 p.m. except in the 8 h time course study which started at 10 a.m. and ended at 6 p.m.

2.2.2. Gastric emptying measurement.

Measurement of gastric emptying was performed as described previously (Taché et al., 1987, Sütő et al., 1994b). Briefly, 1.5 ml of solution containing 1.5% methylcellulose (Sigma Chemical Co., St. Louis, MO) and 0.05% phenol red (Sigma Chemical Co., St. Louis, MO) was given intragastrically through oral intubation with a stainless steel tube to conscious rats. Rats were killed by inhalation of carbon dioxide 20 min later. In each experiment, a rat was killed immediately after intragastric administration of the test solution. Stomachs were clamped at the pylorus and cardia ends, removed, and rinsed in 0.9% saline. Stomachs were then placed in 100 ml of 0.1 N NaOH, and homogenized for 30 s. The suspension was allowed to settle for 60 min at room temperature, and 5 ml of the supernatant was added to 0.5 ml of

20% trichloroacetic acid. After centrifugation (2.800 rpm, for 20 min) 4 ml of 0.5 N NaOH was added to the supernatant. The absorbance of the samples was read at a wavelength of 560 nm by spectrophotometer (Shimadzu UV-260). The gastric emptying for each rat was calculated according to the following formula:

gastric emptying (%) = (1 -absorbance of test sample/ absorbance of baseline) x 100.

2.2.3. Drugs and treatments.

Injections into the jugular vein (i.v.) or into the cisterna magna (i.c.) were performed in rats under short (2.5 min) enflurane anaesthesia. Rats given i.c. injections were placed in a stereotaxic instrument. The volumes of i.c. and i.v. injections were 10 μl/rat and 0.15 ml/rat respectively.

Human recombinant IL-1β (Upjohn, Kalamazoo, MI) was dissolved in Ca^{++}/Mg^{++} free Dulbecco's phosphate buffered saline (pH 7.8). Samples were aliquoted at a concentration of 1 μg/10 μl and stored at -70 °C. Immediately before administration, the stock solution of IL-1β was diluted in 0.9% saline for i.c. injection or in 0.1% serum bovine albumin and 0.9% saline for i.v. injection. IL-1β was injected i.c. (0.01, 0.1, or 1 ng/rat) or i.v. (0.01, 0.1, 1, 3, 6, or 10 ng/rat) 30 min before the administration of the non-caloric solution, except in one experiment where the solution was given at 5, 30, 60, 180 or 480 min after i.c. IL-1β (0.1 ng/rat).

Interleukin-1 receptor antagonist (IL-1ra) (Upjohn, Synergen, Boulder, CO) was dissolved in 0.9% NaCl and was aliquoted at the concentration of 1 μg/10 μl. Aliquots were

stored at -70 °C, and diluted by 0.9% NaCl before the experiments. IL-1ra or vehicle (10 μl) was injected i.c. at a dose of 100 ng/rat in 10 μl immediately before i.c. injection of IL-1β (0.1 ng/10 μl) or vehicle (10 μl).

Indomethacin (Sigma Chemical Co., St. Louis, MO) was dissolved in 1% sodium bicarbonate, and was injected intraperitoneally (i.p.) at 5 mg/kg in 0.5 ml volume 60 min before i.c. injection of IL-1β (0.1 ng/rat) or vehicle.

The recently developed CRF antagonist, [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ and rat CRF (supply by Dr. J. Rivier, the Salk Institute, La Jolla, CA) were synthesized and purified as previously described (Hernandez et al., 1993). Peptides were kept in powder form at -20 °C and immediately before the experiment, CRF was dissolved in saline and the CRF antagonist in distilled water (pH 7.0, warmed to 37° C). [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (10-30 μg/rat in 10 μl) or vehicle (10 μl) was injected i.c. immediately before the i.c. injection in 10 μl of CRF (600 ng/rat), IL-1β (0.1 ng/rat) or vehicle.

2.2.4. Statistical analysis.

Results are expressed as means ± SEM. Comparisons between two groups were calculated by Student's t test. Multiple group comparisons were performed by analysis of variance followed by Dunnett's contrast. A P value < 0.05 was considered statistically significant.

2.3. Results.

Control groups injected with saline either i.c. or i.v. had a gastric emptying rate of 57.4% ± 2.5% and 53.7% ± 1.8%

respectively as measured 20 min after the intragastric administration of the methylcellulose phenol red solution in conscious rats (Fig. 2.1.). IL-1ß injected either i.c. (0.01-1 ng/rat) or i.v. (0.1-10 ng/rat) 30 min before the intragastric solution induced a dose-related inhibition of gastric emptying (Fig. 2.1.). The median effective dose (ED_{50}) was 0.1 and 3 ng/rat for i.c. and i.v. administration, respectively. The i.c. IL-1ß dose of 1 ng and the i.v. dose of 10 ng inhibited gastric emptying by 81.5% and 88.5% respectively (Fig. 2.1.). Time course studies showed that i.c. IL-1ß (0.1 ng/rat) injected either 5, 30, 60, 180, 360 and 480 min before the intragastric administration of the solution induced a similar decrease of gastric emptying (30.3% ± 4.8%, 27.3% ± 3.7%, 32.2% ± 3.9%, 29.0% ± 5.2%, and 28.8% ± 6.4%, respectively). However, gastric emptying was no longer inhibited when measured 480 min after i.c. injection of IL-1β (0.1 ng/rat) (Fig. 2.2.). In rats injected i.c. with vehicle, gastric emptying was not different during the time course study, except, when measured after 8 hours (Fig. 2.2.). Gastric emptying was 71.4% ± 4.8% when assessed 480 minutes after vehicle injection under short enflurane anesthesia compared with 47.4% ± 6.9% when measured 5 minutes after vehicle injection ($P< 0.05$).

IL-1ra, injected i.c. at 100 ng/rat completely prevented the inhibitory effect of IL-1β (0.1 ng/rat, i.c.) on gastric emptying (Fig. 2.3.). When given alone, IL-1ra did not influence basal gastric emptying (Fig. 2.3.).

I.c. injection of CRF (600 ng) and IL-1ß (0.1 ng) inhibited gastric emptying by 69.7% and 62.3% respectively (Fig. 2.4.). The newly developed CRF antagonist, [DPhe^{12},Nle^{21,38},C$^{\alpha}$MeLeu^{37}]CRF_{12-41} injected i.c. at doses of 10 or 20 μg had no effect on basal gastric emptying (gastric emptying: vehicle, 61.4% ± 4.5%; CRF

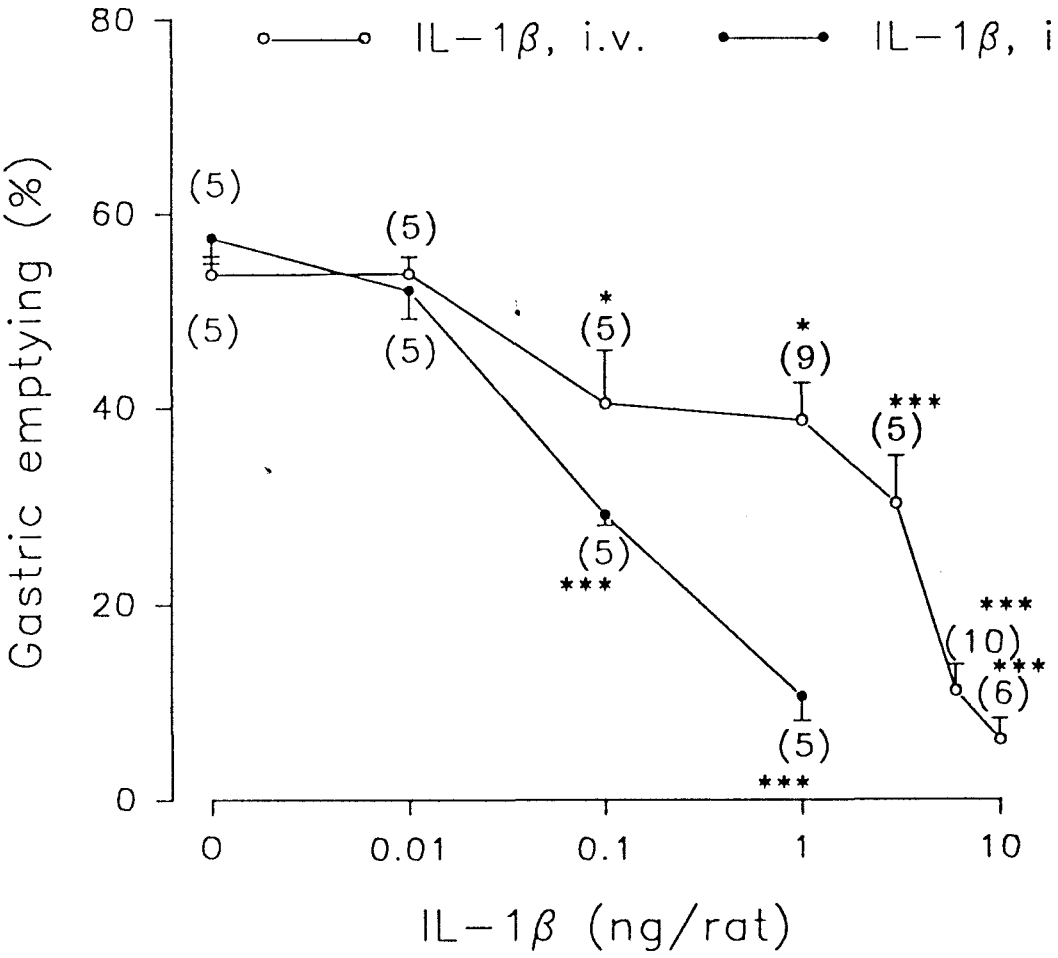

Fig. 2.1. Dose-related inhibition of gastric emptying by i.c. or i.v. injection of IL-1ß in conscious rats. Rats under short enflurane anesthesia were injected with vehicle or IL-1ß at various doses and 30 min later, the 20 min rate of gastric emptying was measured. Each point represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 and ***= P<0.001 compared respective i.c. or i.v. vehicle-treated group.

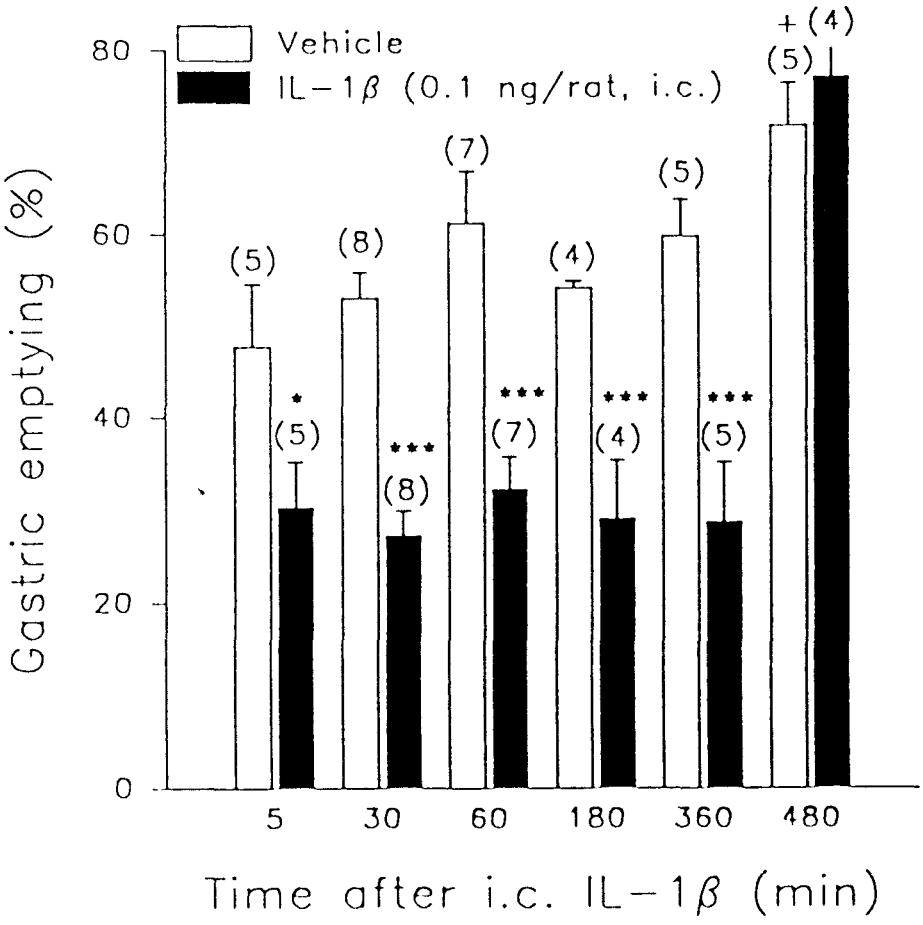

Fig. 2.2. Time course inhibition of gastric emptying induced by i.c. IL-1β in conscious rats. IL-1β or vehicle was given i.c. under short enflurane anesthesia and 5, 30, 60, 180, 360 or 480 min later, the 20 min rate of gastric emptying rate was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= $P<0.05$; ***= $P<0.001$ compared with respective vehicle-treated group; += $P<0.05$ compared with 5 min vehicle group.

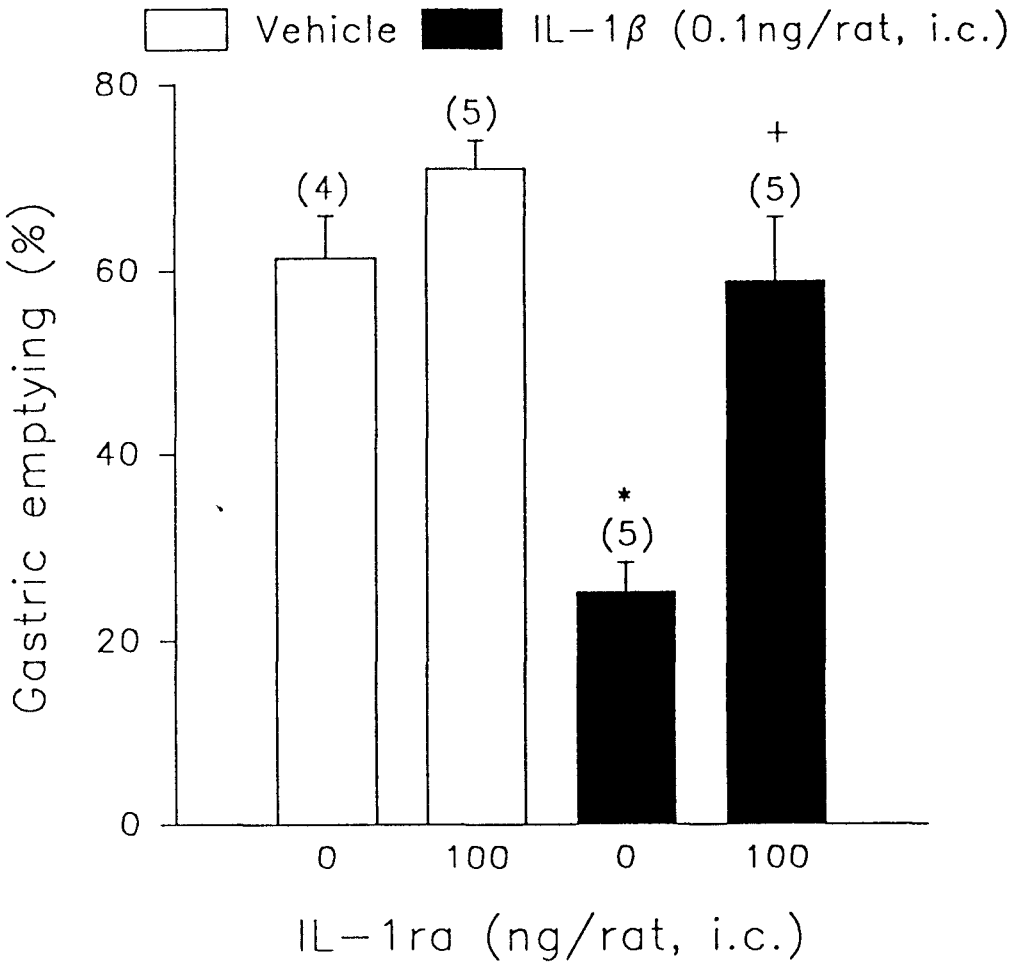

Fig. 2.3. Reversal by i.c. IL-1 receptor antagonist of i.c. IL-1-induced delayed gastric emptying in conscious rats. IL-1ra or vehicle was injected i.c. before IL-1ß or vehicle under short enflurane anesthesia and 30 min later, gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with vehicle-treated group, += P<0.05 compared with respective control group.

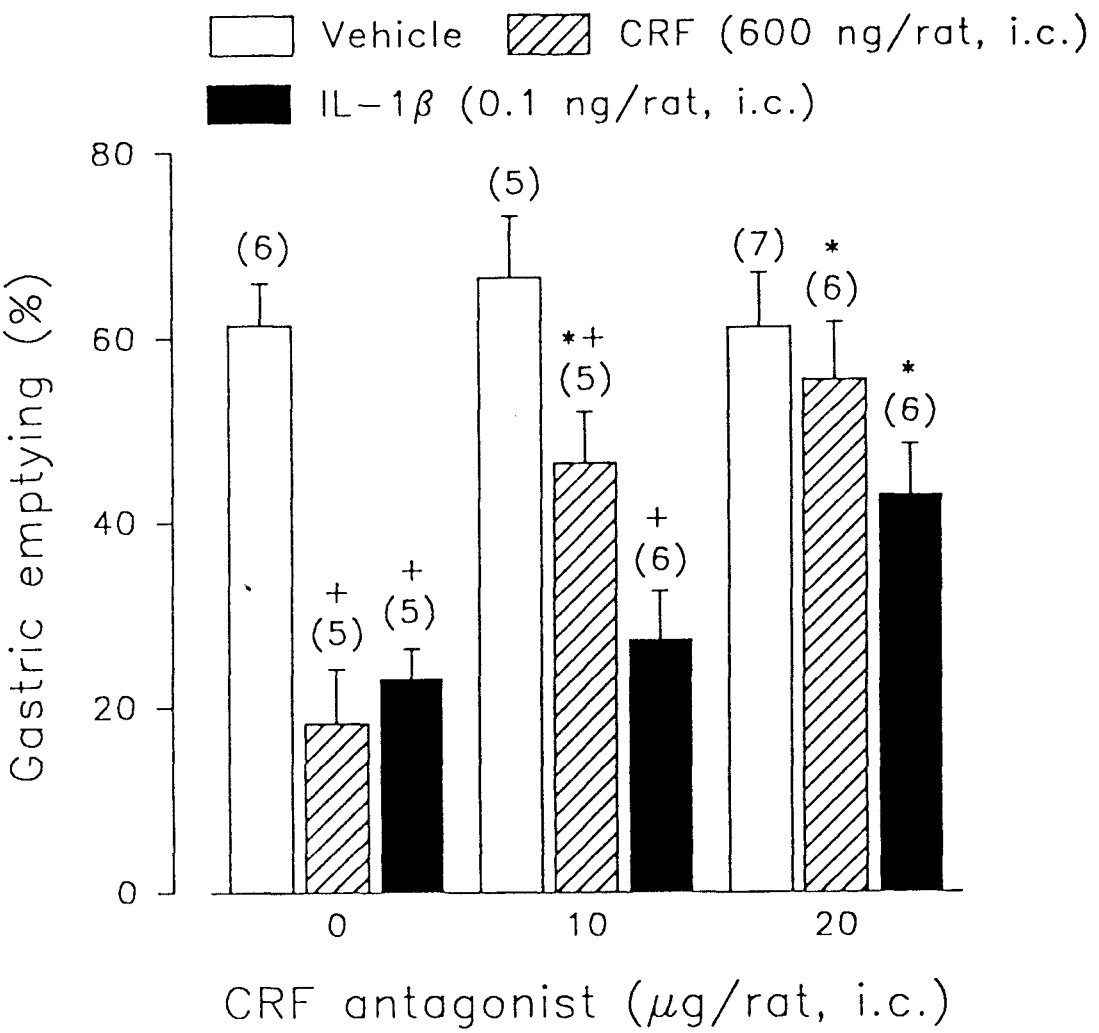

Fig. 2.4. Influence of CRF antagonist injected i.c. on i.c. CRF- or IL-1ß-induced delayed gastric emptying. The CRF antagonist, [DPhe^{12}, Nle^{21,38},C$^{\alpha}$MeLeu^{37}]CRF$_{12-41}$, or vehicle was injected i.c. immediately before i.c. injection of vehicle, CRF or IL-1ß (0.1 ng/rat) under short enflurane anesthesia. Gastric emptying was measured 30 min later. Each point represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with CRF or IL-1ß alone group, += P<0.05 compared with respective vehicle treated group.

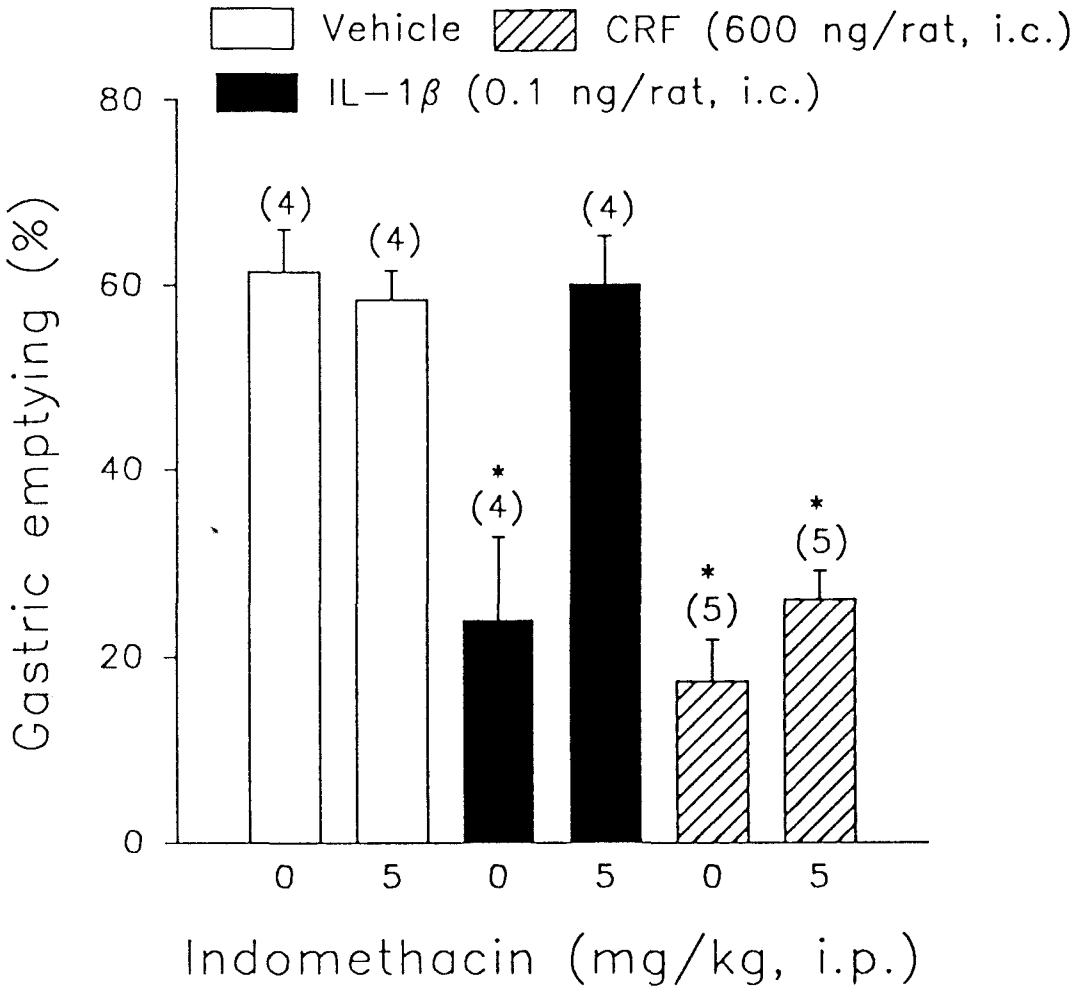

Fig. 2.5. Reversal by indomethacin of i.c. IL-1ß-induced delayed gastric emptying. Indomethacin or vehicle was given i.p. 60 min before i.c. IL-1ß or vehicle and 30 min later gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with vehicle-treated group.

analog (10 μg): 61.4% ± 6.6%; 20 μg: 61.1% ± 5.8%). At the i.c. dose of 20 μg, the CRF antagonist reversed the inhibitory effect of i.c. CRF by 86.8% CRF and that of i.c. IL-1ß (Fig. 2.4.) by 52%. A higher dose of the CRF analog (30 μg, i.c.) given alone decreased gastric emptying by 27% (44.4% ± 3.1%, n=6, $P<0.05$, data not shown).

Inhibition of gastric emptying induced by 0.1 ng i.c. IL-1β was completely eliminated by indomethacin pretreatment (5 mg/kg, i.p.) whereas indomethacin had no effect on gastric stasis elicited by CRF (600 ng, i.c.) (Fig. 2.5.). There was no significant difference between the magnitude of the inhibtory effect of i.c. CRF (17.4% ± 4.4%) and i.c. IL-1β (23.9% ± 8.9%). Indomethacin injected alone did not influence basal gastric emptying (Fig. 2.5.).

2.4. Discussion.

IL-1β (0.01-1 ng) injected into the cerebrospinal fluid at the level of the cisterna magna dose dependently delayed gastric emptying of a non-caloric test meal in conscious rats. The potency of i.c. IL-1ß is shown by the long lasting inhibition of gastric emptying induced by femtomole doses. A time-course study was performed by varying the time intervals between the IL-1 (injected i.c. at the ED_{50}) and the oral administration of test meal. IL-1β inhibitory action was shown to have a short onset (within 20 min), to persist over 6 h and to be reversible because 8 h after the injection, gastric emptying was no longer inhibited. Likewise, the inhibition of gastric acid secretion (Uehara et al., 1989; Saperas et al., 1990) and gastroprotection against ethanol-induced lesions (Robert et al., 1991b) induced by i.c. injection of IL-1 were reported to last more than 6

hours. The slow degradation of IL-1 in the brain (Banks et al., 1991b) and the ability of IL-1ß to stimulate its own IL-1 gene expression in different tissues (Walter et al., 1989; Wang et al., 1991) may account for the prolonged biological activities of IL-1ß. These findings indicate that IL-1β injected i.c. potently inhibits not only gastric secretory (Okumura et al., 1990; Taché et al., 1992b) but also motor function in conscious rats.

Previous studies have shown that i.p. injection of IL-1ß delays gastric emptying of a solid meal in conscious rats (Robert et al., 1991a; McCarthy et al., 1992). In addition, biologically active IL-1 occurs in the systemic circulation following i.c. injection of IL-1ß. These data raise the possibility that i.c. IL-1 can delay gastric emptying through leakage to the periphery. To ascertain that i.c. IL-1 acts in the brain, dose responses of IL-1ß injected i.v. and i.c. were compared under the same conditions. i.v. injection of IL-1ß (0.1-10 ng) dose dependently inhibits gastric emptying of liquid non caloric meal by 0-88.5%. These results and previous findings indicate that peripheral injection of IL-1ß (i.v. or i.p.) delays gastric emptying of both non nutrient solution and solid caloric meals (Robert et al., 1991a; McCarthy et al., 1992). However, the ED_{50} dose of IL-1 given i.c. (0.1 ng/rat) was found to be 30 fold lower than when given i.v. (3 ng/rat). Moreover, the increased levels in the circulation after i.c. injection of IL-1ß were observed at i.c. doses ranging from 100 to 500 ng which are 10^3 higher than the i.c. dose biologically active to inhibit gastric emptying. In addition it is unlikely that circulating IL-1 inhibits gastric emptying through a direct action on gastric smooth muscles because IL-1β potentiates the myotropic activity of $CaCl_2$ and PGE_2 on rat stomach strips (Mugridge et al., 1991). These data

indicate that i.c. IL-1β acts in the brain to inhibit gastric emptying as shown for the inhibition of gastric acid secretion (Saperas et al., 1990; Taché et al., 1992b; Saperas et al., 1992b). Preliminary studies indicate that IL-1ß microinjected into the dorsal vagal complex inhibits stimulated gastric motility in anesthetized rats (Morrow et al., 1995). IL-1 microinjected into the PVN, preoptic area and anterior hypothalamus was previously shown to inhibit gastric acid secretion in conscious rats (Saperas et al., 1992b). These hypothalamic sites, namely the PVN of the hypothalamus are activated after i.c. or i.v. injection of IL-1 as shown by c-fos expression (Ericsson et al., 1994; Ju et al., 1991; Rivest et al., 1992, Ericsson et al., 1997). Chemical or electrical stimulation of the PVN of the hypothalamus inhibits gastric motor function (Sakagushi et al., 1985; Monnikes et al., 1992) suggesting hypothalamic and medullary possible sites of action for central IL-1 to inhibit gastric emptying.

The central action of IL-1β is expressed through specific interaction with IL-1 receptors. The recently identified natural IL-1ra binds to surface cell receptors of IL-1 (Carter et al., 1990; Eisenberg et al., 1990; Hannum et al., 1990; Arend, 1991; Dinarello et al., 1991). When administered at 10^3 excess, IL-1ra blocked the biological activities of IL-1β in various in vivo or in vitro studies (Dinarello et al., 1991; Saperas et al., 1992a; Saperas et al., 1993). Likewise, in the present study, IL-1ra administered at doses 10^3-fold higher than IL-1β, completely prevented i.c. IL-1β-induced gastroparesis. IL-1ra alone did not significantly modify gastric emptying. This shows the lack of intrinsic activity of IL-1ra which is in agreement with various other evidence (Dinarello et al., 1991). The characterization and expression of IL-1 receptors showed that in the brain, IL-1

receptors are mainly of type I (80-kilodalton protein) (Farrar et al., 1987; Cunningham et al., 1992; Cunningham et al., 1993) which is preferentially recognized by IL-1ra (Dinarello et al., 1991). These data suggest that i.c. IL-1ß may act through IL-1 receptor type I.

IL-1β has the ability to stimulate prostaglandin release in several cell types including astrocytes and to modulate the activity of brain neurons through prostaglandin dependent pathways (Hori et al., 1988; Hartung et al., 1989; Nakashima et al., 1989; Shibata et al., 1990). Central IL-1β-induced alterations of thermoregulation and hormone secretion have been ascribed to brain prostaglandin release (Coceani et al., 1988; Taché et al., 1992b). In our experiment, indomethacin injected i.p. at a dose that had no effect on basal gastric emptying, completely prevented the delayed gastric emptying in response to i.c. IL-1β injection. Likewise, i.p. injection of indomethacin reversed the centrally mediated action of IL-1 to induce inhibition of gastric acid secretion and ulcer formation (Uehara et al., 1989; Saperas et al., 1990; Robert et al., 1991b; Shibasaki et al., 1991; Saperas et al., 1992b) and the suppression of postprandial pattern of intestinal motility (Fargeas et al., 1993). These findings suggest that prostaglandin pathways are involved in mediating i.c. IL-1β-induced inhibition of gastric emptying, however the site and mechanisms through which prostaglandins are involved need to be further established.

Convergent evidence suggest that prostaglandin may be involved in the central action of IL-1 partly through modulation of CRF release. First, in vivo and in vitro studies indicate that IL-1 stimulates CRF secretion in the hypothalamus through prostaglandin dependent mechanisms (Uehara et al., 1987; Katsuura et al., 1988b; Bernardini et al., 1990a; Navarra et al., 1991;

Rivier et al., 1991). Second, stimulation of endogenous release of CRF in the brain is well established to inhibit gastric motor function in rats (Taché et al., 1993). Third, the recently developed longer acting CRF antagonist, [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (Hernandez et al., 1993; Howard et al., 1993) injected i.c. was able to reverse by 52% the inhibition of gastric emptying induced by i.c. IL-1. Likewise, i.c.v. IL-1β-induced stimulation of colonic motor function was shown to be partially mediated by brain CRF pathways (Fargeas et al., 1993). The CRF antagonist was used at a 20 μg i.c. dose that antagonized i.c. CRF-induced delayed gastric emptying while having no intrinsic activity. These data further established that the CRF antagonist is about twofold more potent than α-helical CRF$_{9-41}$ in its ability to reverse exogenous and endogenous CRF-induced inhibition of gastric emptying, as reported in other in vivo systems (Hernandez et al., 1993; Howard et al., 1993). However, at a higher dose, (30 μg/rat, i.c.), [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ showed agonist activity. These data suggest that prostaglandins may act by modulating CRF release, as previously established (Uehara et al., 1987; Katsuura et al., 1988b; Bernardini et al., 1990a; Navarra et al., 1991; Rivier et al., 1991) rather than by altering CRF action. In the present study, i.c. injection of IL-1β-induced gastric stasis was partially reversed by i.c. injection of a CRF antagonist, whereas indomethacin given at a dose that suppressed prostaglandin synthesis in both the brain and the stomach has been shown to provide a full blockade (Saperas et al., 1991). These findings suggest that prostaglandin may also exert a direct central action to influence gastric emptying independently of the one mediated by CRF release. PGE_2 injected into the CSF, PVN of the hypothalamus, or preoptic area suppresses gastric acid secretion in rats independently of CRF

(Barocelli et al., 1991; Saperas et al., 1991; Saperas et al., 1992b) and modulates intestinal migrating motor complexes in dogs (Staumont et al., 1990). So far all drugs shown to act centrally to inhibit gastric acid secretion also delay gastric emptying (Taché et al., 1990a; Taché et al., 1990b). However, whether central injection of PGE_2 also influences gastric motor function is still unknown.

The possible pathophysiological relevance of these findings is supported by neuroanatomical and biological observations. IL-1 immunoreactivity and receptors are localized in the brain (Giulian et al., 1986; Farrar et al., 1987; Breder et al., 1988; Hetier et al., 1988; Katsuura et al., 1988a; Lechan et al., 1990; Lee et al., 1993; Takao et al., 1990; Cunningham et al., 1993). IL-1 production is increased in the brain in response to immune challenge or injury (Higgins et al., 1991; Yan et al., 1992). There is also evidence that, systemic IL-1β reaches the brain through a saturable, carrier mediated transport system (Banks et al., 1989; Reimers et al., 1991; Banks et al., 1991a) and activates CRF neurons in the PVN of the hypothalamus (Saphier et al., 1990; Ju et al., 1991; Watanabe et al., 1991; Lee et al., 1993). Because the IL-1 antagonist injected i.c. did not alter basal gastric emptying, these data suggest that under basal conditions, central IL-1 does not regulate gastric motor function. However, the potent inhibition of gastric emptying induced by i.c. injection of IL-1ß may have implications for the alteration of gastric motor function during immunologic challenges associated with activation of IL-1 release.

3. Intravenous interleukin-1β-induced inhibition of gastric emptying: Involvement of central corticotrophin-releasing factor and prostaglandin pathways in rats[2].

3.1. Background.

Interleukin-1 (IL-1) is a cytokine which is synthesized primarily by effector cells of the immune system. IL-1 was originally described as a modulator of immune response during inflammation and injury (Dinarello, 1988). Compelling evidence suggests that IL-1 is not only involved in the communication of immune cells but affects non-immune organ systems, in particular the neuroendocrine system in the brain (Rothwell et al., 1994). Peripheral or central injection of IL-1ß activates corticotropin-releasing factor (CRF) neurons in the PVN of the hypothalamus (PVN) and the release and synthesis of hypothalamic CRF (Sapolsky et al., 1987; Bernardini et al., 1990a; Saphier et al., 1990; Ju et al., 1991; Navarra et al., 1991; Watanabe et al., 1991). There is evidence that prostaglandin may be part of the mechanisms mediating hypothalamic CRF release (Katsuura et al., 1988b; Bernardini et al., 1990a; Katsuura et al., 1990; Navarra et al., 1991).

Exogenous injection of CRF into specific brain sites and endogenous activation of brain CRF pathways by stress are well established for their role in altering gastrointestinal function (Taché et al., 1993). In particular, CRF injected into the

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cerebrospinal fluid or PVN or activation of CRF neurons in the PVN by various stressors inhibits gastric emptying and stimulates colonic motor function through an action mediated by the autonomic nervous system (Taché et al., 1993). This is independent of the endocrine effect of the peptide (Taché et al., 1993). Likewise, recent studies showed that IL-1ß delivered into the cerebrospinal fluid (CSF) inhibits gastric emptying of a non nutrient semi-liquid meal while stimulating colonic motility through central CRF dependent mechanisms in rats (Fargeas et al., 1993; Sütő et al., 1994b). Peripheral administration of IL-1ß either intraperitòneally or intravenously is also known to inhibit gastric emptying of solid meal or a methylcellulose solution (Robert et al., 1991a; McCarthy et al., 1992; Sütő et al., 1994b). However, the mechanisms through which peripheral administration of IL-1ß suppresses gastric emptying are still not known. Since peripheral injection of IL-1ß stimulates PVN neurons containing CRF as shown by c-fos expression in this nuclei (Chan et al., 1993; Ericsson et al., 1994; Brady et al., 1994) and CRF release (Watanabe et al., 1991), gastric stasis induced by peripheral IL-1ß may involve central prostaglandin mediated CRF release. In addition, we previously established that IL-1ß is more potent when injected intracisternally (i.c.) than intravenously (Sütő et al., 1994b, Chapter 2.).

In the present study, the receptor specificity of intravenous IL-1ß-induced inhibition of gastric emptying was investigated using the selective interleukin-1 receptor antagonist (IL-1ra) injected intravenously and intracisternally (Dinarello et al., 1991; Arend, 1991). Possible involvement of CRF and prostaglandin pathways in the gastric response to peripheral injection of IL-1ß was also explored.

3.2. Materials and methods.

3.2.1. Animals.

Male Sprague-Dawley rats (Harlan Sprague Dawley, San Diego, CA) weighing 250-300 g, were maintained under controlled housing conditions of light (6.00 to 18.00 h), humidity (35%-55%), and temperature (21 ± 2°C) for one week. Food and water were available ad libitum. Rats were deprived of food for 18 h before the experiments but had access to water up to the time when gastric emptying was measured. All experiments were performed between 12.00. and 16.00 h

3.2.2. Gastric emptying measurement.

Gastric emptying was measured as described previously (Sütő et al., 1994b). Briefly, 1.5 ml of a solution containing 1.5% methylcellulose (Sigma Chemical Co., St. Louis, MO) and 0.05% phenol red (Sigma Chemical Co., St. Louis, MO) was given intragastrically through orogastric intubation with a stainless steel tube to conscious rats. Rats were killed by inhalation of carbon dioxide 20 min later. In each experiment, a rat was killed immediately after intragastric administration of the test solution. Stomachs were clamped at the pylorus and cardia ends, removed, and rinsed in 0.9% saline. Stomachs were then placed in 100 ml of 0.1 N NaOH, and homogenized for 30 s. The suspension was allowed to settle for 60 min at room temperature, and 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid. After centrifugation (2.800 rpm, for 20 min) 4 ml of 0.5

N NaOH was added to the supernatant. The absorbance of the samples was read at a wavelength of 560 nm by spectrophotometer (Shimadzu UV-260). The gastric emptying for each rat was calculated according to the following formula:

gastric emptying (%) = (1 -absorbance of test sample/absorbance of baseline) x 100.

3.2.3. Drugs and treatments.

Injections into the jugular vein (i.v.) or into the cisterna magna (i.c.) were performed in rats under short (2.5 min) enflurane anaesthesia. For intracisternal injections rats were placed in a stereotaxic instrument. The volumes of intracisternal and intravenous injections were 10 μl/rat and 0.15-0.2 ml/rat, respectively.

Human recombinant IL-1β (Upjohn, Kalamazoo, MI) was dissolved in Ca^{++}/Mg^{++} free Dulbecco's phosphate buffered saline (Ph 7.8). Samples were aliquoted at a concentration of 1 μg/10 μl and stored at -70 °C. Immediately before administration, the stock solution of IL-1β was diluted in 0.1% serum bovine albumin and 0.9% saline for intravenous injection. In all experiments, except the time course study, IL-1β was injected i.v. at the ED_{50} (3 ng/rat) 30 min before the administration of phenol red methylcellulose solution as previously established (Sütő et al., 1994b).

Human recombinant interleukin-1 receptor antagonist (IL-1ra) (Synergen, Boulder, CO) was dissolved in 0.9% NaCl and was aliquoted at the concentration of 1 μg/10 μl. Aliquots were stored at -70°C, and diluted by 0.9% NaCl before the experiments.

IL-1ra was injected intracisternally (100 ng/rat) or intravenously (3 μg/rat) immediately before vehicle or IL-1β. The selection of the intracisternal and intravenous doses of IL-1ra were based on our previous studies showing the reversal of intracisternal IL-1ß (0.1 ng)-induced inhibition of gastric emptying under similar conditions (Sütő et al., 1994b) as well as the established 10^3:1 ratio of peripheral IL-1ra:IL-1ß to inhibit the biological actions of peripheral IL-1 (Dinarello et al., 1991; Saperas et al., 1992a; Saperas et al., 1993). Control rats were given vehicle either under the same conditions. Indomethacin (Sigma Chemical Co., St. Louis, MO) was dissolved in 1% sodium bicarbonate, and was injected intraperitoneally (i.p.) at 5 mg/kg in 0.5 ml volume 60 min before i.v. IL-1β or vehicle.

The recently developed CRF antagonist, [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (supplied by Dr. J. Rivier, the Salk Institute, La Jolla, CA) was synthesized and purified as previously described (Hernandez et al., 1993). The peptide was kept in powder form at -20 °C and dissolved in distilled water (Ph 7.0, warmed to 37°C) immediately before the experiment. [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (20 μg/rat) or vehicle was injected intracisternally or intravenously followed by that of IL-1β or vehicle. The choice of the dose of CRF antagonist given intracisternally was based on our previous reports showing the blockade of exogenous and endogenous CRF-induced inhibition of gastric emptying (Sütő et al., 1994b; Hernandez et al., 1993). In an additional experiment, [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (20 μg/rat) was injected i.v. immediately before i.v. injection of rat CRF (600 ng, supplied by Dr. J. Rivier, Salk Institute, CA) and 30 min later the phenol red methylcellulose solution was administered.

3.2.4. Statistical analysis.

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 Dunnett's contrast. A P value < 0.05 was considered statistically significant.

3.3. Results.

The 20 min rate of gastric emptying of the phenol red methylcellulose solution measured 30 min after i.v. injection of vehicle under short enflurane anesthesia was 53.7% ± 1.9% (n=12). IL-1ß (3 ng/rat) injected intravenously 30 min before the orogastric delivery of the solution decreased gastric emptying to 30.3% ± 4.8% (n=12, P<0.05). IL-1ra injected intravenously (3 μg/rat) or intracisternally (100 ng/rat) immediately before that of IL-1ß (3 ng/rat, i.v.) prevented by 100% and 62% respectively the inhibition of gastric emptying induced by IL-1β (Fig. 3.1.). IL-1ra given alone either intravenously or intracisternally under otherwise similar conditions had no effect on basal gastric emptying (Fig. 3.1.).

Indomethacin (5 mg/kg, i.p.) did not influence the basal rate of gastric emptying in vehicle-pretreated group, but completely abolished intravenous IL-1β-induced inhibition of gastric emptying (Fig. 3.2.). The recently developed CRF antagonist, [DPhe^{12},Nle^{21,38},C$^{\alpha}$MeLeu^{37}]CRF$_{12-41}$ (20 μg/rat) injected intracisternally or intravenously did not significantly modify gastric emptying in vehicle-treated rats (Fig. 3.2.). The CRF

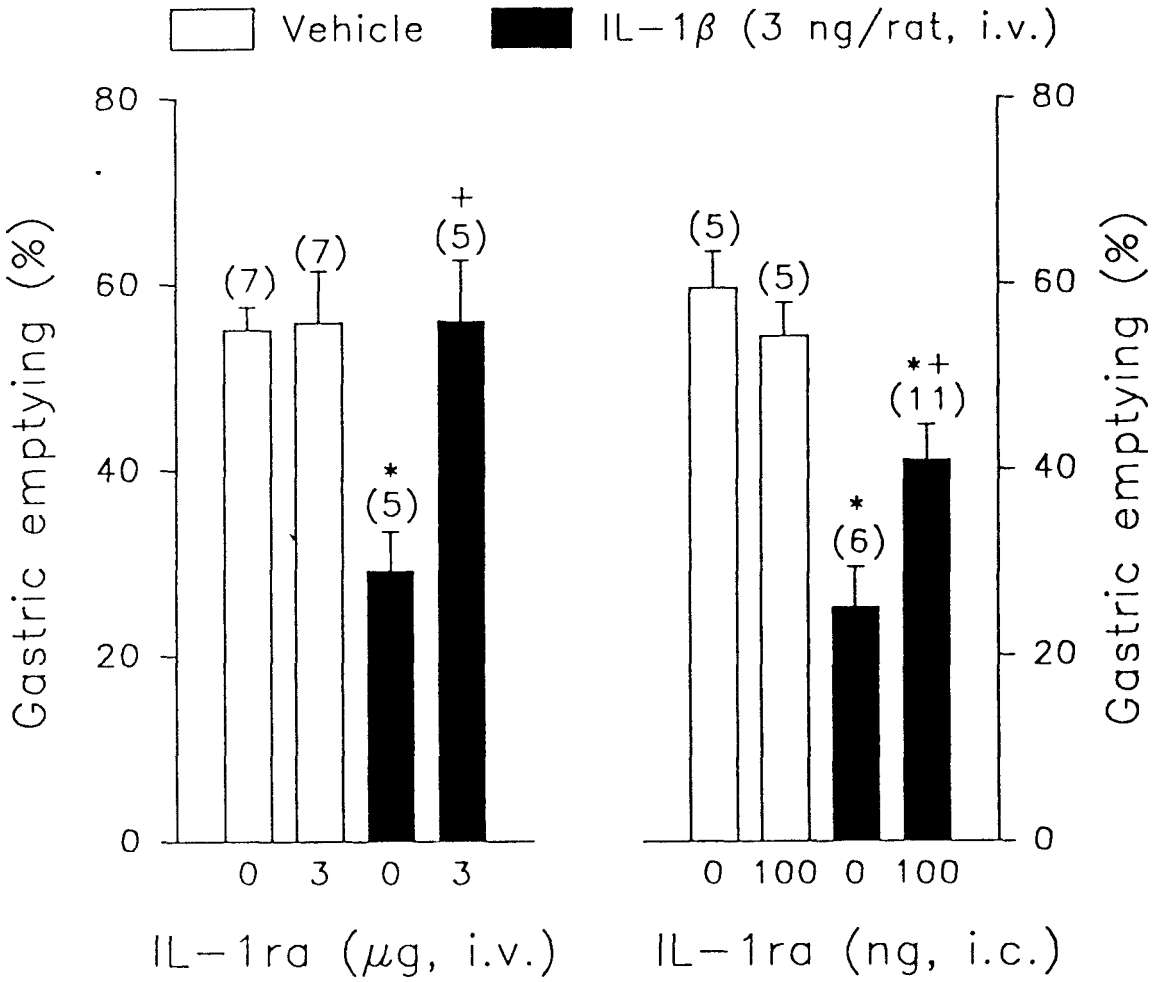

Fig. 3.1. Inhibition of intravenous IL-1β induced delay of gastric emptying by intravenous or intracisternal injection of IL-1 receptor antagonist in conscious rats. IL-1ra or vehicle was injected intravenously or intracisternally immediately before intravenous IL-1β or vehicle under short enflurane anesthesia and 30 min later, gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with vehicle-treated group, += P<0.05 compared with respective IL-1β-treated group.

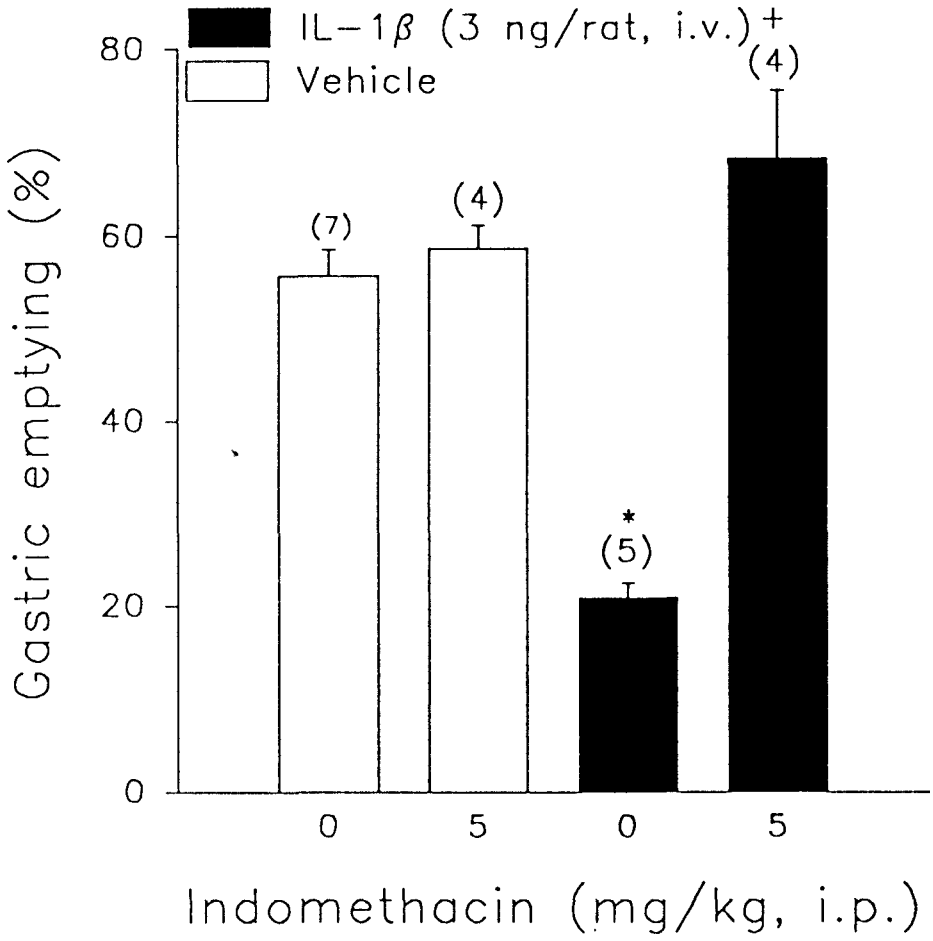

Fig. 3.2. Reversal by indomethacin of intravenous IL-1ß-induced delayed gastric emptying. Indomethacin or vehicle was given intraperitoneally 60 min before intravenous IL-1ß or vehicle and 30 min later gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with vehicle-treated group, += P<0.05 compared with respective IL-1ß-treated group.

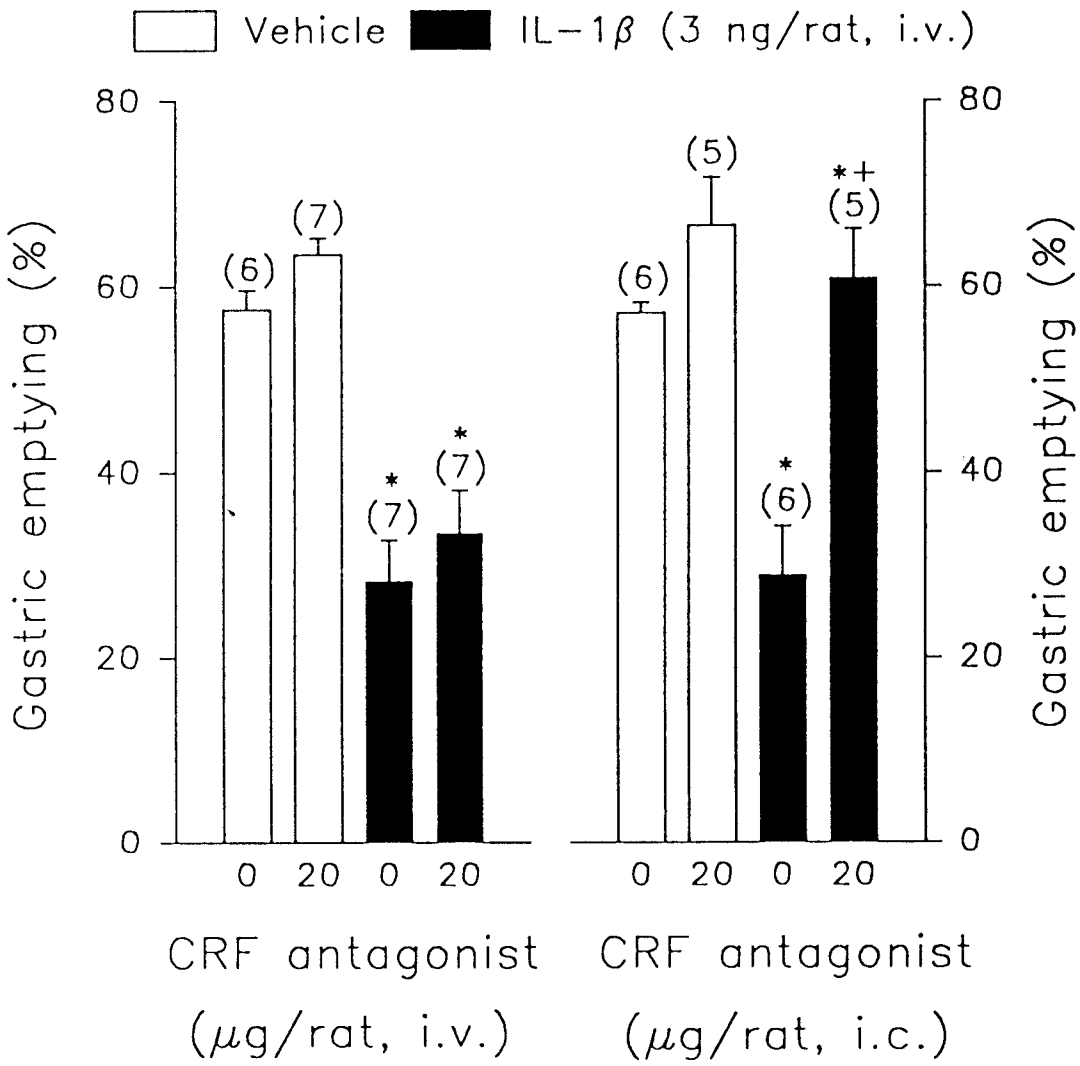

Fig. 3.3. Influence of CRF antagonist injected i.v. or i.c. on i.v. IL-1ß-induced delayed gastric emptying. The CRF antagonist, [DPhe^{12}, Nle^{21,38},C$^{\alpha}$MeLeu^{37}]CRF$_{12-41}$, or vehicle was injected i.v. or i.c. immediately before injection of vehicle or IL-1ß under short enflurane anesthesia. Gastric emptying was measured 30 min later. Each point represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with respective vehicle-treated group += P<0.05 compared with respective IL-1ß-treated group.

antagonist (20 μg/rat) injected intravenously did not alter intravenous IL-1ß-induced 50.6% inhibition of gastric emptying (Fig. 3.3.) but completely prevented intravenous CRF (600 ng/rat)-induced 58% inhibition of gastric emptying (vehicle + CRF: 23.3% ± 3.3%, n=4; CRF antagonist + CRF: 71.3% ± 1.3%, n=4; $p<0.05$). By contrast, the CRF antagonist (20 μg/rat) injected intracisternally completely abolished the inhibitory effect of intravenous IL-1ß (Fig. 3.3.).

3.4. Discussion.

Human recombinant IL-1ß injected intravenously at 3 ng dose 30 min before administration of the non-caloric solution inhibits the 20 min rate of gastric emptying by 50% in conscious rats as previously described (Sütő et al., 1994b, Chapter 2.). IL-1ß and IL-1α injected intraperitoneally were also reported to inhibit postprandial gastric emptying in conscious rats (Robert et al., 1991a; McCarthy et al., 1992) with an ED_{50} dose of 105 ng/rat (Robert et al., 1991a). These data indicate that peripheral administration of IL-1 consistently delays gastric emptying of both solid nutrient meal and non-nutrient solution.

IL-1ra binds to IL-1 receptors and blocks the effects of exogenous IL-1ß at doses 10^{2-3} fold higher than that of IL-1ß (Arend, 1991; Saperas et al., 1993). IL-1ra injected intravenously completely abolished the delay in gastric emptying induced by intravenous IL-1ß at a $1:10^3$ ratio. These results indicate that exogenous IL-1ß inhibits gastric motor function through specific interaction with IL-1 receptors. Similar blockade of i.p. IL-1ß-induced postprandial gastric stasis was observed when IL-1ra was injected i.p. at a 1:300 ratio (Robert

et al., 1991a).

The mechanisms whereby IL-1ß injected into the circulation inhibits gastric emptying through IL-1 receptor mediated events are poorly understood. Potential action directly on smooth muscles is unlikely since in isolated rat stomach strip preparation, IL-1ß potentiates the myotropic activity of $CaCl_2$ (Mugridge et al., 1991). In addition, the relaxing effect of IL-1ß on rat gastric fundus strips is not altered by indomethacin (Montuschi et al., 1994). By contrast, in the present study, indomethacin completely prevented intravenous IL-1ß-induced delayed gastric emptying. Several biological actions of peripherally administered IL-1ß have shown to be centrally mediated (Rothwell, 1991; Terao et al., 1993). The present data and available neuroanatomical evidence are consistent with a possible involvement of brain pathways in the delayed gastric emptying induced by circulating IL-1ß.

We previously established that the ED_{50} of IL-1ß inhibiting gastric emptying is 30 fold lower when the cytokine is injected intracisternally than intravenously (Sütő et al., 1994b, Chapter 2.). Second, IL-1ra (100 ng) injected intracisternally reduced the response evoked by intravenous injection of IL-1ß by 46%. This dose of IL-1ra given intracisternally was previously shown to reverse completely the 60% inhibition of gastric emptying-induced by intracisternal injection of IL-1ß (0.1 ng=ED_{50}). The partial reversal obtained under the present conditions may reflect incomplete accessibility of the IL-1ra to brain sites of action reached by IL-1ß given peripherally (Banks et al., 1989; Hashimoto et al., 1991; Banks et al., 1991a; Banks et al., 1991b; Reimers et al., 1991). Alternatively, the partial reversal may also result in the use of subeffective intravenous IL-1ß:intracisternal IL-1ra ratio (1:33). A 200-1000-fold excess of

the IL-1ra is required to obtain a complete reversal of IL-1 actions by the IL-1ra (Arend, 1991; Dinarello et al., 1991; Saperas et al., 1993; Rothwell et al., 1994). The appearance into the circulation of biologically active IL-1ß when injected into the cisterna magna at doses superior to 100 ng (Robert et al., 1991b) precluded testing higher doses of the IL-1ra by this route. Since IL-1ra and IL-1ß share striking similar sequences (Dinarello et al., 1991), leakage of the IL-1ra antagonist into the circulation may occur as observed for IL-1ß (Robert et al., 1991b).

Our present and previous studies (Sütő et al., 1994b, Chapter 2.) show a similarity between mediators (prostaglandin and CRF) that are involved in the gastric stasis induced by both central and peripheral routes of IL-1ß administration. Indomethacin given i.p. at 5 mg/kg which inhibits brain and gastric PGE_2 generation (Saperas et al., 1991), abolished IL-1ß-induced inhibition of gastric emptying when injected either intracisternally (Sütő et al., 1994b, Chapter 2.) or intravenously (present study). By contrast, ibuprofen and indomethacin injected i.p. (10 mg/kg) were reported to prevent partly or to have no effect on the delay in gastric emptying of a solid meal induced by IL-1 injected i.p. at the maximal effective dose (Robert et al., 1991a; McCarthy et al., 1992). These discrepancies may be related to differences in the experimental protocols (non nutrient liquid, vs. nutrient solid meal, ED_{50} vs maximal IL-1ß doses). Although IL-1ß injected i.p. increased PGE_2 formation in the gastric mucosa which is blocked by indomethacin (Robert et al., 1991a), it is unlikely that indomethacin action results from the local inhibition of PGE_2 in the stomach. Intravenous injection of PGE_2 did not influence gastric emptying in rats while 16,16,dimethyl PGE_2 injected

intravenously or subcutaneously stimulated gastric emptying (Ruwart et al., 1984). In addition the relaxing effect of IL-1ß on rat gastric fundus strips is not modified by indomethacin (Montuschi et al., 1994).

Indomethacin injected i.p. at doses of 7-10 mg/kg prevents the activation of CRF-ACTH release induced by intravenous IL-1ß (Ruwart et al., 1984; Katsuura et al., 1988b; Bernardini et al., 1990; Kaatsuura et al., 1990) suggesting that peripheral IL-1ß-induced PGE_2 release in the brain is involved in the activation of hypothalamic CRF pathways. Intravenous injection of IL-1ß increases the firing rate of CRF-containing neurons as well as the synthesis and release of the peptide or c-fos expression in the PVN (Saphier et al., 1990; Watanabe et al., 1991; Chover-Gonzalez et al., 1993; Ericsson et al., 1994, Ericsson et al. , 1997). CRF released endogenously by various stressors or central injection of CRF acts in the PVN and the dorsal motor nucleus of the vagus (DMN) to inhibit gastric motor function through autonomic pathways (Taché et al., 1993). We previously showed that intracisternal injection of CRF-induced delayed gastric emptying is not altered by peripheral administration of indomethacin (Sütő et al., 1994b, Chapter 2.). In the present study, the delay of gastric emptying induced by intravenous IL-1ß was completely prevented by the newly developed CRF antagonist, [DPhe^{12},Nle^{21,38},C$^{\alpha}$,MeLeu^{37}]-CRF_{12-41} (Hernandez et al., 1993) injected intracisternally but not peripherally. Taken together the previous and present data suggest that gastric stasis resulting from peripheral injection of IL-1ß may involve a central nervous system mediated release of prostaglandin and CRF. The exact brain sites at which peripherally injected IL-1ß acts to induce an indomethacin- and CRF-dependent inhibition of gastric emptying are still to be investigated. The PVN has been shown to be a

responsive site to exogenous and/or endogenous CRF-induced inhibition of gastric motor function and to IL-1ß-induced suppression of gastric acid secretion [38-40]. In addition, recent studies showed that intravenous injection of IL-1ß produces a robust and long lasting c-fos mRNA response in the autonomic-related parts of the parvocellular PVN neurons and in identified CRF producing cells in the PVN (Chan et al., 1993; Ericsson et al., 1994; Brady et al., 1994, Ericsson et al., 1997).

While the route whereby intravenous IL-1 comes to act in the brain remain unsettled (Rothwell et al., 1994), several mechanisms have been unravelled. There is evidence that systemic IL-1 can enter the CSF and brain parenchyma in intact form by a saturable, carrier mediated system and that the entry is blocked by IL-1ra (Banks et al., 1989; Banks et al., 1991a; Banks et al., 1991b; Reimers et al., 1991; Banks et al., 1993). Recent reports showing considerable IL-1 uptake by endothelial brain capillaries and high concentrations of IL-1 type I receptor mRNA in the endothelial cells of postcapillary venules throughout the rat brain (Wong et al., 1994) suggest that capillary beds, in addition to circumventricular organs, may be sites through which peripheral IL-1 gains entry into the brain (Banks et al., 1993; Brady et al., 1994).

IL-1ra injected intravenously or intracisternally did not influence the rate of gastric emptying of a non-caloric solution in control rats (Sütő et al., 1994b, Chapter 2, present study). Likewise, intraperitoneal injection of IL-1ra did not alter the postprandial rate of the gastric emptying in rats (Robert et al., 1991a). These results indicate that IL-1ß is not involved in the regulation of gastric emptying under basal conditions as previously reported for brain CRF (Taché et al., 1993). However

peripheral IL-1ß production is increased during infection, inflammatory processes or stress (Ulich et al., 1990; Korneva et al., 1992) suggesting a possible role of elevated circulating IL-1ß in the alterations of gastric motor function encountered during immune challenge (McCarthy et al., 1992). The 3 ng intravenous dose of IL-1ß inhibiting gastric emptying is well within the range found in the circulation in response to endotoxin challenge (Derijk et al., 1992).

The present data further support the interactions between the immune system and neuronal regulation of gastric motor function which may have implications in the understanding of gastric stasis associated with pathologies activating IL-1ß release. It also strengthens the role of brain CRF in the gastric stasis resulting not only from psychological, physical or chemical stress (Taché et al., 1993) but also from immunological challenge increasing circulating IL-1.

4. Endotoxin inhibits gastric emptying through peripheral release of corticotropin releasing factor and interleukin-1β.

4.1. Background.

The local inflammation and systemic symptoms of infection induced by gram negative bacteria are caused by the lipopolysaccharide constituent (LPS) of the bacterial wall. The integrated endocrine, neural and immune response to a foreign antigen challenge, is generally accepted as the acute phase reaction (Kushner, 1982; Cybulsky et al., 1988). Many components of the symptoms associated with the acute phase response results from the disturbed function of the gastrointestinal tract: e.g. nausea, vomiting, abdominal fullness. Endotoxin injection into experimental animals mimics many of these symptoms, and alters different gastrointestinal functions including gastric acid secretion (Saperas et al., 1994), gastric mucosal blood flow and mucosal integrity (Whittle et al., 1987; Pique et al., 1988), intestinal motility (Esplugues et al., 1989; Pons et al., 1989), colonic water and electrolyte transport (Ciancio et al., 1992).

LPS is a potent stimulus of the activation of the immune system. LPS injection into experimental animals results in the release of cytokines interleukin-1β (IL-1β) and tumor necrosis alpha (TNFα) (Sirko et al., 1989; Zuckerman et al., 1989; Ulich et al., 1990), arachidonic acid metabolites (Sirko et al., 1989), platelet-activating factor (Whittle et al., 1987), nitric oxide (Nava et al., 1992), calcitonin gene-related peptide (CGRP) (Griffin et al., 1992; Wang et al., 1992b; Hüttemeier et al., 1993), opioid peptides (Hamilton et al., 1986; Ulich et al., 1990). Recent studies indicate that the action of LPS is mediated

at least partly by the CNS. LPS was found to activate the hypothalamic-pituitary-adrenal axis (Rivier et al., 1989a; Long et al., 1990), to suppress plasma gonadotropin levels (Long et al., 1990; Rivier, 1990), to induce IL-1β within the CNS (Koenig et al., 1990; Hillhouse et al., 1993; Wan et al., 1993) and other peripheral sites (Ulich et al., 1990) as well.

IL-1β injected intracisternally (i.c.) or intravenously (i.v.) was found to inhibit gastric emptying (Sütő et al., 1994b; Sütő et al., 1996, Chapter 2., present study). The mechanism stimulated by both central an peripheral injection of IL-1β utilizes the medullary release of prostaglandin and corticotropin releasing factor (CRF) (Sütő et al., 1994b; Sütő et al., 1996, Chapters 2., 3.).

CRF injected i.c. stimulates autonomic pathways mediated inhibition of gastric emptying (Taché et al., 1987). Many locations of CRF also were established outside the CNS such as peripheral T and B lymphocytes (Stephanou et al., 1990), testes (Dufau et al., 1993), trophoblastic and decidual tissue (Petraglia et al., 1990), pancreas (Petrusz et al., 1983) different types of cancers (Suda et al., 1984a) and the human gastrointestinal tract (Kawahito et al., 1994; Kruseman et al., 1982), or capsaicin sensitive primary afferent neurons (Skofitsch et al., 1984; Kamilaris et al., 1992). CRF injected not only i.c. but i.v. delays gastric emptying (Taché et al., 1987; Sheldon et al., 1990).

The aims of the present study were to evaluate the role of central and peripheral interleukin-1β and CRF in the gastroparesis elicited by intraperitoneal administration of specific receptor antagonists and prostaglandin synthesis inhibitor indomethacin.

4.2. Materials and methods.

4.2.1. Animals.

Male Sprague-Dawley rats (Harlan Sprague Dawley, San Diego, CA) weighing 250-300 g, were maintained under controlled housing conditions of light (6 a.m. to 6 p.m.), humidity (35%-55%), and temperature (22 ± 2°C) for one week. Food and water were available ad libitum. Rats were deprived of food for 18 h before the experiments but had access to water up to the time where gastric emptying was measured. All the experiments were performed between 12 p.m. and 4 p.m. except in the 8 h time course study which started at 10 a.m. and ended at 6 p.m.

4.2.2. Gastric emptying measurement.

Measurement of gastric emptying was performed as described previously (Sütő et al., 1994b). Briefly, 1.5 ml of solution containing 1.5% methylcellulose (Sigma Chemical Co., St. Louis, MO) and 0.05% phenol red (Sigma Chemical Co., St. Louis, MO) was given intragastrically through oral intubation with a stainless steel tube to conscious rats. Rats were killed by inhalation of carbon dioxide 20 min later. In each experiment, a rat was killed immediately after intragastric administration of the test solution. Stomachs were clamped at the pylorus and cardia ends, removed, and rinsed in 0.9% saline. Stomachs were then placed in 100 ml of 0.1 N NaOH, and homogenized for 30 s. The suspension was allowed to settle for 60 min at room temperature, and 5 ml

of the supernatant was added to 0.5 ml of 20% trichloroacetic acid. After centrifugation (2.800 rpm, for 20 min) 4 ml of 0.5 N NaOH was added to the supernatant. The absorbance of the samples was read at a wavelength of 560 nm by spectrophotometer (Shimadzu UV-260). The gastric emptying for each rat was calculated according to the following formula:

gastric emptying (%) = (1 -absorbance of test sample/ absorbance of baseline) x 100.

4.2.3. Drugs and treatments.

Injections into the jugular vein (i.v.) or into the cisterna magna (i.c.) were performed in rats under short (2.5 min) enflurane anesthesia. Rats given i.c. injections were placed in a stereotaxic instrument. The volumes of i.c., i.v. or i.p. injections were 10 μl/rat, 0.15 ml/rat or 2 ml/kg respectively.

LPS was injected at different doses (0.075, 0.75, 7.5, 15, 75, μg/kg) intraperitoneally. The gastric emptying was measured 60 min later. Three other group of animals were treated by 7.5 μg/kg LPS and the gastric emptying was measured 30, 60 or 480 min later. Respective control groups were treated by i.p. saline (0.5 ml).

Interleukin-1 receptor antagonist (IL-1ra) (Upjohn, Synergen, Boulder, CO) was dissolved in 0.9% NaCl and was aliquoted at the concentration of 1 μg/10 μl. Aliquots were stored at -70 °C, and diluted by 0.9% NaCl before the experiments. IL-1ra or vehicle (10 μl) was injected i.c. or i.v. at a dose of 100, 500 ng/rat or 3 μg/rat in 10 μl immediately

before i.p. injection of LPS (7.5 μg/kg) or vehicle (10 μl).

The recently developed CRF antagonist, [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ and rat CRF (supply by Dr. J. Rivier, the Salk Institute, La Jolla, CA) was kept in powder form at -20 °C and immediately before the experiment it was dissolved in distilled water (pH 7.0, warmed to 37° C). [DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$ (10-30 μg/rat in 10 μl) or vehicle (10 μl) was injected i.c. or i.v. immediately before the i.p. injection of LPS (7.5 μg/kg) or vehicle. Indomethacin (Sigma Chemical Co., St. Louis, MO) was dissolved in 1% sodium bicarbonate, and was injected intraperitoneally (i.p.) at 5 mg/kg 60 min before i.p. injection of LPS (7.5 μg/kg) or vehicle.

4.2.4. Statistical analysis.

Results are expressed as means ± SEM. Comparisons between two groups were calculated by Student's t test. Multiple group comparisons were performed by analysis of variance followed by Dunnett's contrast. A P value < 0.05 was considered statistically significant.

4.3. Results.

Control groups injected with saline i.p. had a gastric emptying rate 65.7% ± 4.5% measured 60 min later. LPS injected at doses of 0.075, 0.75, 7.5, 15, 75, μg/kg, i.p. dose dependently decreased gastric emptying (61.2% ± 8.8%, 40.8% ± 5.4%, 31.0% ± 10.0%, 12.6% ± 2.5%, 6.9% ± 2.9%, respectively) (Fig. 4.1.). The gastric emptying 30, 60, 480 min after LPS injection (7.5 μg/kg, i.p.) was 51.7% ± 7.6%, 13.6% ± 2.45%, 29.5% ± 2.7%, respectively ($P<0.05$) (Fig. 4.2.). The respective control groups had an emptying rate of 54.9% ± 2.6%, 61.1% ± 2.1% and 72.5% ± 5.0%.

IL-1ra injected both i.v. (3 μg/rat) or i.c. (500 ng/rat) did not influence basal gastric emptying rate (57.8% ± 3.4% vs. 61.0% ± 0.9% i.v., 58.4% ± 4.9% vs. 54.6% ± 3.9% i.c.) (Fig. 4.3.) Intravenous IL-1ra at a dose as low as 100 ng/rat fully reversed the inhibition of gastric emptying induced by LPS (7.5 μg/kg, i.p.) (27.5% ± 4.4% vs. 57.7% ± 15.3%, $P<0.05$) (Fig. 4.3.). The further increase of the dose of i.v. IL-1ra (500 ng/rat, 3 μg/rat) did not influence the gastric emptying rate (55.2% ± 5.8% and 54.9% ± 8.2%, respectively). IL-1ra injected i.c. at doses of 100, 300 and 500 ng/rat resulted in a dose dependent increase of gastric emptying, but the inhibition of gastroparesis was not complete (29.8% ± 3.6% vs. 32.9% ± 7.3%, 38.24% ± 11.2%, 45.22% ± 6.4%, respectively).

CRF antagonist injected either i.v. (20 μg/rat) or i.c. (20 μg/rat) did not influence the gastric emptying of saline treated rats (59.9% ± 2.3% vs. 59.16% ± 2.1% i.v., 60.7% ± 5.2% vs. 69.6% ± 3.3% i.c.) (Fig.4.4.). However the inhibition of gastric emptying was abolished after i.v. injection of CRF antagonist (20 μg/rat) (27.6% ± 2.6% vs. 54.3% ± 2.1%, $P< 0.05$), the i.c.

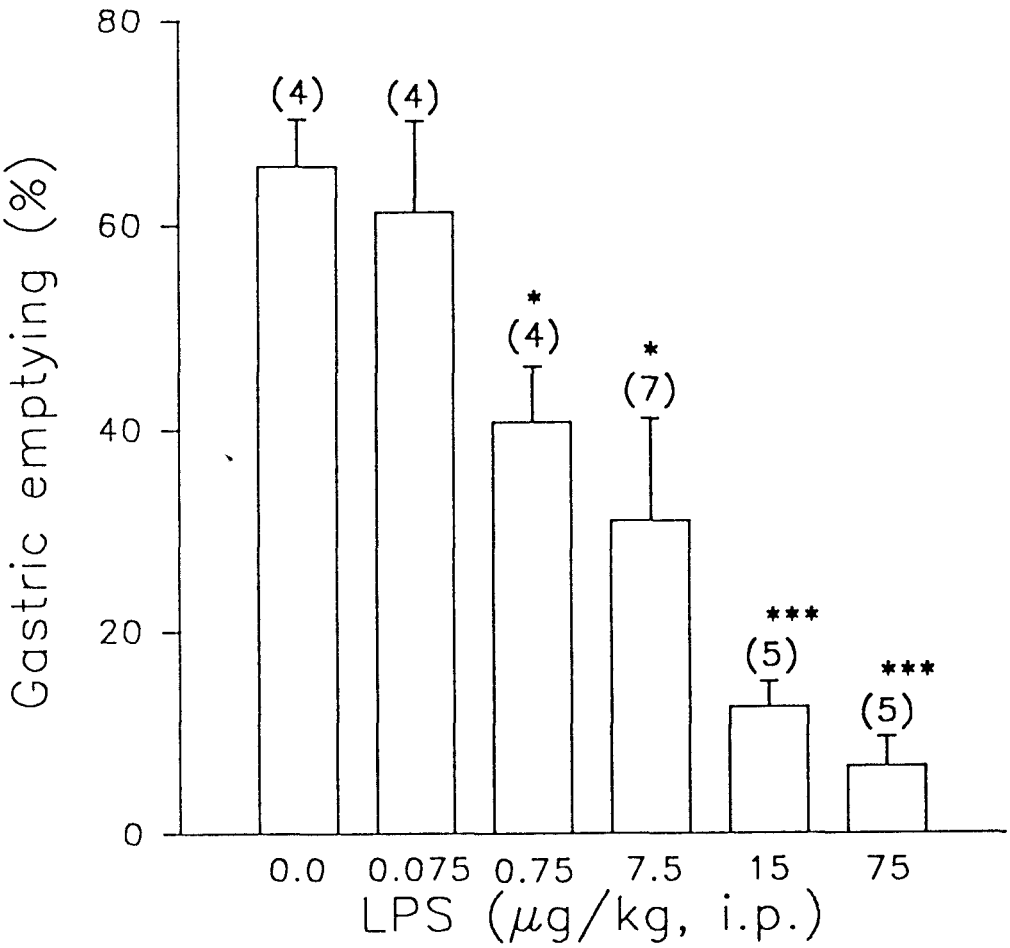

Fig. 4.1. Dose related inhibition of gastric emptying by i.p. injection of LPS in coscious rats. Rats under short enflurane anesthesia were injected with vehicle or LPS at various doses and 60 min later, the 20 min rate of gastric emptying was measured. Each point represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 and ***= P<0.001 compared with vehicle treated group.

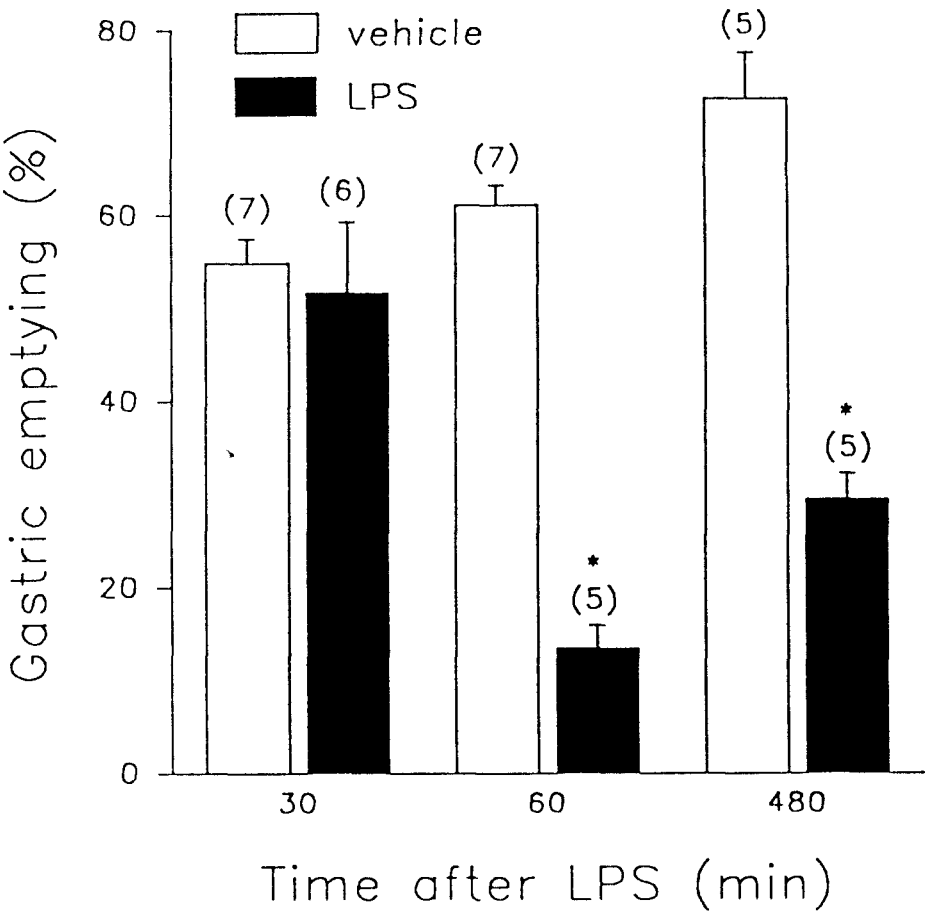

Fig. 4.2. Time course inhibition of gastric emptying induced by i.p. LPS in conscious rats. LPS or vehicle was given i.p. and 30, 60 or 480 min later, the 20 min rate of gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with respective vehicle-treated group.

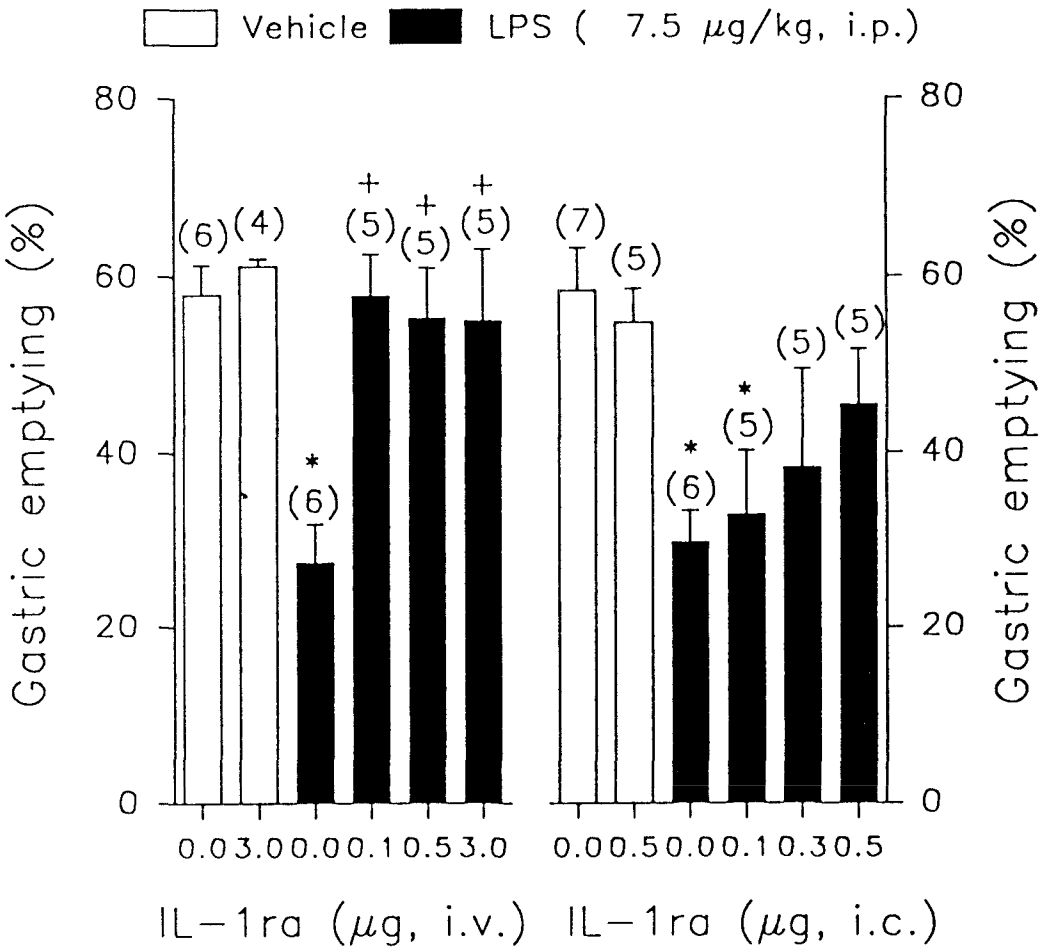

Fig. 4.3. Inhibition of i.p. LPS-induced delay of gastric emptying by i.c. or i.v. injection of IL-1ra in conscious rats. IL-1ra or vehicle was injected i.v. or i.c. immediately before i.p. LPS or vehicle under short enflurane anesthesia and 30 min later, the 20 min rate of gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= $P<0.05$ compared with vehicle-treated group, += $P<0.05$ compared with respective LPS-treated group.

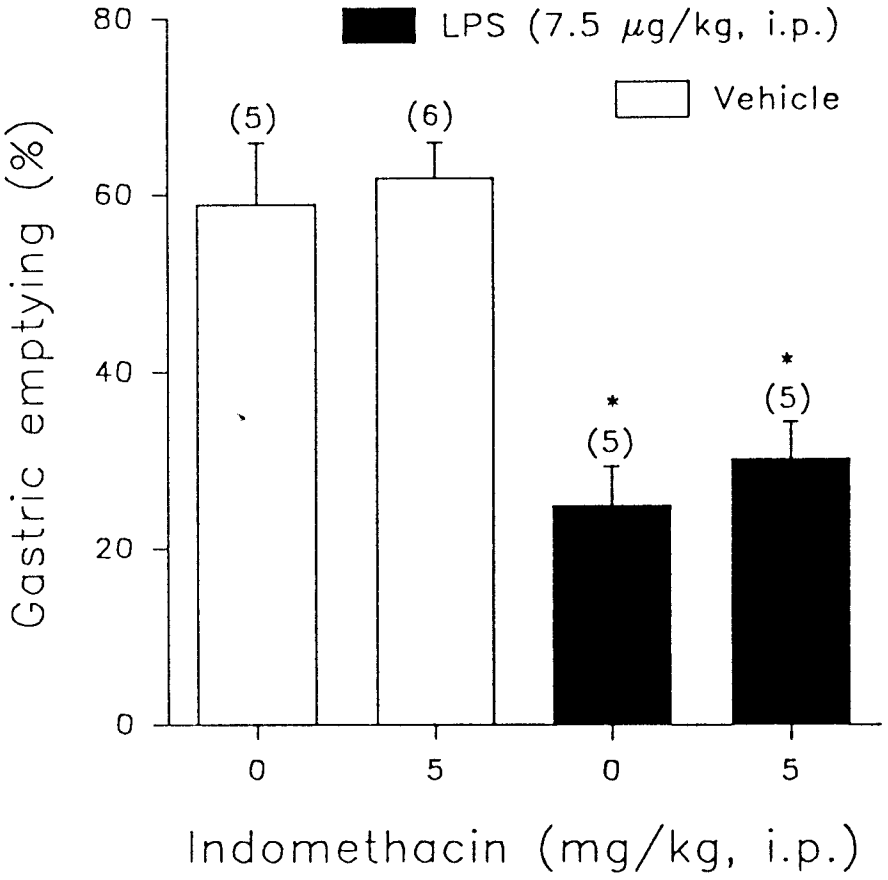

Fig. 4.4. Effect of indomethacin on i.p. LPS-induced delay of gastric emptying. Indomethacin or vehicle was given i.p. 60 min before i.p. LPS or vehicle and 30 min later, the 20 min rate of gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. * = $P<0.05$ compared with vehicle-treated group.

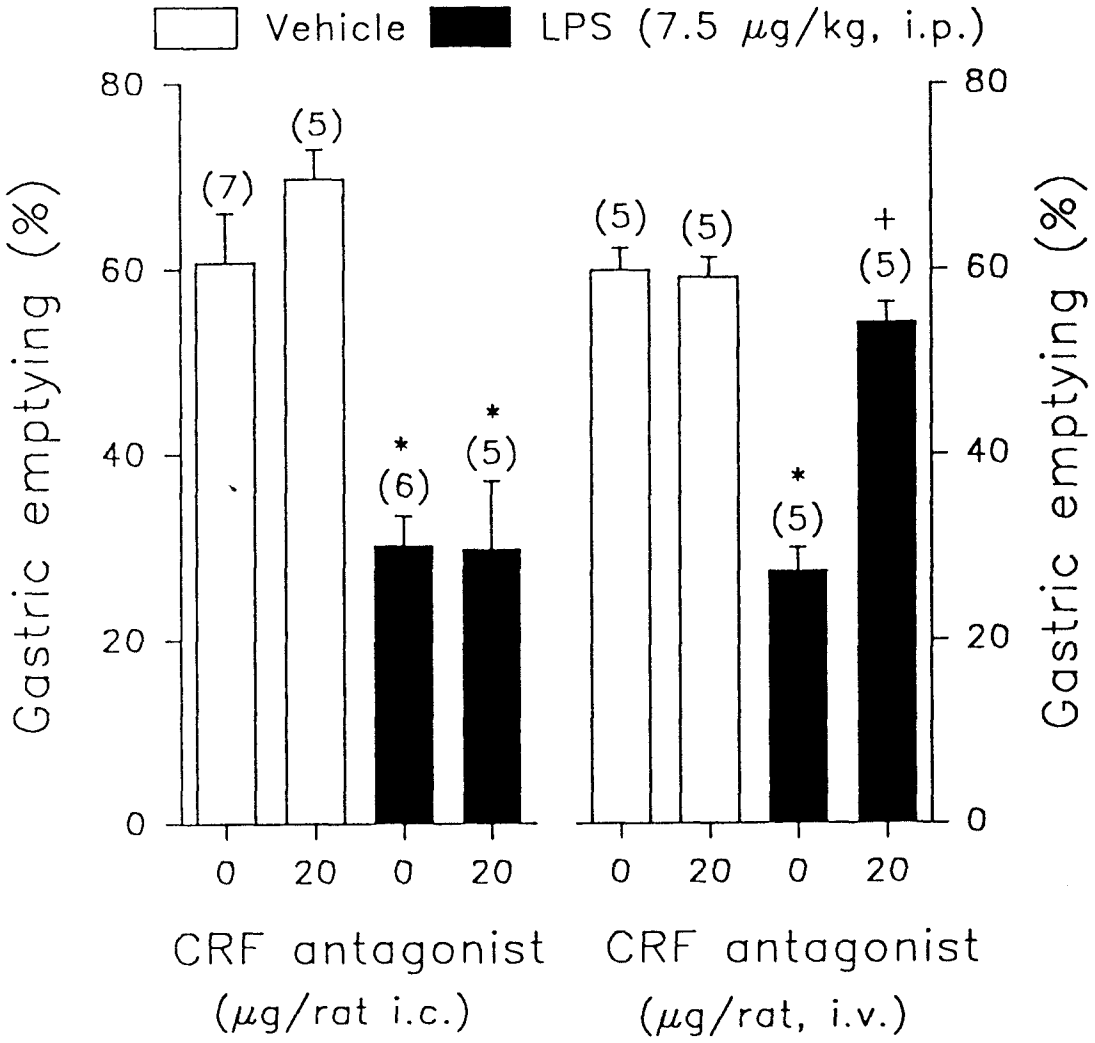

Fig. 4.5. Influence of CRF antagonist injected i.c. or i.v. on i.p. LPS-induced delayed gastric emptying. The CRF antagonist, [DPhe^{12}, Nle^{21,38},C$^{\alpha}$MeLeu^{37}]CRF$_{12-41}$, or vehicle was injected i.c. or i.v immediately before i.p. injection of vehicle or LPS under short enflurane anesthesia. The 20 min rate of gastric emptying was measured 30 min later. Each point represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with respective vehicle-treated group , += P<0.05 compared with respective LPS treated group.

injection of CRF antagonist (20 μg/kg) did not influence the gastric emptying (30.4% ± 3.1% vs. 29.9% ± 7.2%) (Fig. 4.4.).

Indomethacin (5 mg/kg, i.p.) has no influence on the gastric emptying of rats injected with either saline (0.5ml, i.p.) (58.9% ± 7.0% vs. 61.8% ± 4.0%) or LPS (7.5 μg/kg, i.p.) (24.9% ± 4.3% vs. 30.1% ± 4.2%) (Fig 4.5.)

4.4. Discussion.

Infection with gram negative bacteria results in the activation of the host defense mechanisms. The phenomenon of acute phase response (Kushner, 1982) is mediated by various immune, endocrine and neural mechanisms. The injection of LPS, part of the outer layer of gram negative microbes, into experimental animals evokes responses similar to gram negative infection. LPS injected intraperitoneally dose dependently inhibited gastric emptying of noncaloric testmeal 1 h after its administration in conscious rats. The effect of LPS was long lasting (up to 8 h) and was not observed 30 min after i.p. injection of LPS. In response to LPS administration peripheral blood mononuclear cells (PBMCs) produce different cytokines that are the mediators of most actions of endotoxin (Cybulsky et al., 1988; Ulich et al., 1990). The actions of LPS are supposed to be secondary to the release of different cytokines (Cybulsky et al., 1988; Long et al., 1990; Ulich et al., 1990; Ban et al., 1992; Dunn, 1992; Ebisui et al., 1992; Saper et al., 1994). The delay of onset of LPS mediated inhibition of gastric emptying may be due to the synthesis/release of these second mediators.

One of the most proximal component of the immune cascade is IL-1 existing in two forms of alpha and beta (Dinarello, 1988).

LPS (Rivier et al., 1989a; Rivier, 1990) and IL-1 share many biological actions, including the activation of the hypothalamic pituitary adrenal axis, and suppression of the gonadal functions. LPS was shown to induce hypothalamic (Hillhouse et al., 1993) and pituitary IL-1β (Koenig et al., 1990). IL-1β injected both centrally (Sütő et al., 1994b, Chapter 2.) or peripherally (Robert et al., 1991b, Sütő et al., 1996, Chapter 3.) delays gastric emptying through central PG and CRF utilizing pathway.

A specific natural IL-1 receptor antagonist was cloned and expressed which binds to surface cell receptors of IL-1 (Carter et al., 1990; Hannum et al., 1990; Eisenberg et al., 1990; Arend, 1991; Dinarello et al., 1991). This peptide administered at 10^2-10^3-fold excess blocks different biological activities of IL-1 mediated by type I receptors (Dinarello et al., 1991; Saperas et al., 1992a; Saperas et al., 1993). IL-1ra injected i.v. at a dose 100 ng prevented the inhibition of gastric emptying by LPS. When the same dose was injected i.c. it did not influence the gastric emptying delayed by IL-1β. When the dose was further increased up to 500 ng/rat, IL-1ra inhibited the LPS-induced delay of gastric emptying. This action seems to be mediated by the peripheral leak of IL-1ra because IL-1ra and IL-1β share similar amino acid sequences (Dinarello et al., 1991), and IL-1β was shown to leak to the periphery at i.c. doses superior to 100 ng (Wallace et al., 1992). These data have shown that LPS-induced gastroparesis is mediated by peripheral IL-1 acting on type I receptors. IL-1 most likely has a paracrine action since most of the data do not support measurable levels of IL-1 in plasma or blood during gram negative infection. However an endogenous pyrogen activity was observed after execise in human plasma (Cannon et al., 1983) or the interleukin-1 activity of human plasma was increased after ovulation (Cannon et al., 1985),

attempts to measure increase in the concentration of plasma IL-1 were mostly unsuccessful (Dinarello et al., 1984b; Long et al., 1990) during infection. In one experiment a pyrogenic but not subpyrogenic dose of LPS was shown to result in measurable interleukin levels in rat (Derijk et al., 1991) and enhanced plasma levels of IL-1 were only found in patients suffering from critical illness due to infection by gram negative bacteria or in experimental animals injected by sublethal dose of LPS (Moldawer et al., 1987; Zuckerman et al., 1989). A 4.2 kilodalton peptide was isolated from febrile patients, which was identified as a cleavage product of IL-1 functioning as circulating IL-1 activity (Dinarello et al., 1984b).

A specific CRF receptor antagonist was used to test whether LPS inhibits gastric emptying through the release of CRF, because (1.) LPS inhibited gastric emptying through peripheral IL-1β (present study) and i.v. injection of IL-1β was shown to inhibit gastric emptying activating central PG and CRF mediated autonomic pathways, (2.) LPS at low doses was demonstrated to activate CRF containing CNS pathways through IL-1β to stimulate the hypothalamic-pituitary-adrenal axis (Elenkov et al., 1992a; Tilders et al., 1994), (3.) Peripheral injection of endotoxin was shown by c-fos immunochemistry to result in the activation of neurons within the CNS which are involved in the regulation of different autonomic endocrine and behavioral responses during inflammation (Wan et al., 1993; Elmquist et al., 1993). The injection of IL-1β resulted in the similar expression of c-fos suggesting that the neuronal activation elicited by peripheral LPS injection may be mediated by IL-1β (Rivest et al., 1992; Elmquist et al., 1993; Brady et al., 1994; Ericsson et al., 1994; Ericsson et al. 1996). CRF antagonist injected i.c. did not alter the gastroparesis induced by LPS (Navarra et al., 1991). When

injected i.v. prevented the i.p. LPS-induced inhibition of gastric emptying. There are several explanations why this opposite action of CRF antagonist can be observed upon the different routes of administration: (1.) Neurons, other than CRF containing ones are activated by LPS or IL-1β. Both IL-1β (i.v.) and LPS (i.p.) were shown to activate oxytocin and vasopressin (Mouri et al., 1993) immunoreactivity positive neurons within the CNS. Oxytocin is an inhibitory neuropeptide of gastric emptying within the central nervous system (Sütő et al., 1994a). (2.) In response to LPS, other mediators such as IL-6 or TNFα are produced (Cybulsky et al., 1988; Long et al., 1990; Ulich et al., 1990; Dunn, 1992; Foster et al., 1993).

Although central CRF receptor antagonist did not have any effect on LPS inhibited gastric emptying peripheral CRF receptor antagonist prevented the gastroparesis elicited by i.p. LPS. CRF was originally described within the CNS, it was isolated as the primary activator of pituitary corticotrophs originating from the hypothalamus (Vale et al., 1981), but it is localized in other extrapituitary brain sites, and was shown to modulate other autonomic functions independent of HPA axis (Thiefin et al., 1989; La Feuvre et al., 1991). A large pool of CRF was localized thereafter in other brain areas (Suda et al., 1984b) and in other peripheral organs such as peripheral T and B lymphocytes (Stephanou et al., 1990), testes (Dufau et al., 1993), trophoblastic and decidual tissue (Petraglia et al., 1990), pancreas (Petrusz et al., 1983), different types of cancers (Suda et al., 1984a) and the human gastrointestinal tract (Kawahito et al., 1994; Kruseman et al., 1982) as well. CRF is also present in capsaicin sensitive primary sensory afferents (Skofitsch et al., 1984). Peripheral CRF has different functions: delays gastric emptying (Taché et al., 1987), inhibits gastric acid

secretion (Taché et al., 1987), mediates parturition (Petraglia et al., 1990), and it is an antireproductive factor in the testis (Audhya et al., 1989).

The most likely source of peripheral CRF may be represented by peripheral blood mononuclear cells: CRF mRNA was shown in this type of cells which are able to produce CRF-like immunoreactivity (Stephanou et al., 1990). Capsaicin sensitive primary afferents does not seem to be the source of peripheral CRF since in an other series of experiments capsaicin ablation of primary sensory neurones did not influence the delayed gastric emptying by LPS (Chapter 5.).

There are some conflicting results regarding the interaction between peripheral CRF and cytokines originating from PBMCs: (1.) IL-1 was shown to mediate the effect of CRF and AVP on β-endorphin production by human PBMC (Kavelaars et al., 1989). This finding was not confirmed in subsequent studies (Sobel, 1990; Woudenberg et al., 1992). (2.) CRF+AVP or IL-1α/β were shown to stimulate the production of ACTH-like proteins in human mononuclear cells, but the interrelationship between CRF and IL-1 was not examined (Reder, 1992). (3.) A further study elucidated that the presence of endotoxin determines the ability of PBMCs to synthesize IL-1, IL-6 or TNF in response to CRF (Singh et al., 1990; Leu et al., 1992; Pereda et al., 1995).

Prostaglandins, depending on the species being examined, the type of PG and the route of administration, have various effects on the gastrointestinal motility (Sanders, 1984; Dubois et al., 1987; Penston et al., 1989; Nishiyama et al., 1992; Stein et al., 1994). LPS-induced plasma leakage and CGRP release in response to LPS are mediated by PGs (Wallace, 1987), the latter phenomenon is inhibited by indomethacin (Wang et al., 1992a). Peripheral IL-1β-induced inhibition of gastric emptying involves PGs (Chapter

3.). Indomethacin did not alter the gastric emptying in LPS or vehicle treated rats as well. These data demonstrate that PGs do not mediate gastric emptying during basal conditions or after LPS treatment.

Based on these data the most likely mechanism of action of i.p. LPS to delay gastric emptying of a noncaloric testmeal is that LPS induces the peripheral release of CRF and IL-1 through a PG independent pathway. The exact site and target of these peptides needs further investigation, but a peripheral interaction at the level of PBMCs can not be excluded.

5. Non-neural calcitonin gene related peptide contributes to the inhibition of gastric emptying by intraperitoneal endotoxin in rats.

5.1. Background.

The delivery of ingested food from the stomach to the intestine is a well coordinated process resulting in the most effective utilization of different nutrients. Infectious diseases which activate a complex host response consisting of neural, immune and hormonal reactions - this latter called acute phase reaction (Kushner, 1982; Cybulsky et al., 1988)- disturb the upper gastrointestinal motility. Lipopolysaccharide (LPS) -the outer layer of gram negative bacteria- injected into experimental animals elicits similar neural, hormonal and immune reactions to those observed during the development of acute phase reaction. This phenomenon is accompanied by altered gastrointestinal motility (; Esplugues et al., 1989; Pons et al., 1989; Cullen et al., 1995; Wirthlin et al., 1996).

The action of lipopolysaccharide is mediated by different central and peripheral transmitters. It is well established that LPS results in a huge increase of plasma calcitonin gene-related peptide during endotoxic shock (Wang et al., 1991; Griffin et al., 1992; Wang et al., 1992a; Wang et al., 1992b; Hüttemeier et al., 1993). Capsaicin sensitive primary sensory afferents are involved in the of postprandial regulation of the motility of the upper gastrointestinal tract (Holzer et al., 1994; Raybould et al., 1994; Zittel et al., 1994) and in ileus due to surgery and/or peritoneal irritation (Holzer et al., 1986; Holzer et al.,

1992; Plourde et al., 1993b; Takeuchi et al., 1996) in rats. α-CGRP an alternative splicing product of calcitonin gene (Rosenfeld et al., 1983) is abundantly localized within the gastrointestinal tract. Mostly it is stored in and released from primary sensory afferent fibres arising from the dorsal root ganglia, a smaller amount was shown in fibres originating from nodose ganglia (Sternini, 1992). CGRP injected intravenously potently inhibits gastric acid secretion, motility and the development of mucosal lesions (Maggi et al., 1987; Taché, 1991; Taché et al., 1992a). The C terminal fragment of human CGRP, $hCGRP_{8-37}$ is a potent antagonist of CGRP on type 1 receptors (Chiba et al., 1989; Dennis et al., 1990; Donoso et al., 1990). CGRP was shown to inhibit gastric emptying through type 1 receptors, since $hCGRP_{8-37}$ reversed the inhibition of gastric emptying induced by the i.v. injection of CGRP (Plourde et al., 1993a). According to the neuronal localization of CGRP a non neural, source was also described: it is highly likely that gastric mucosal lymphocytes synthesize CGRP mRNA and CGRP as well (Jakab et al., 1993).

The aim of the present study was to establish a role for endogenous CGRP in the inhibition of gastric emptying induced by i.p. LPS injection.

5.2. Materials and methods.

5.2.1. Animals.

Male Sprague-Dawley rats (Harlan Sprague Dawley, San Diego, CA) weighing 250-300 g, were maintained under controlled housing conditions of light (6 a.m. to 6 p.m.), humidity (35%-55%), and

temperature (22 ± 2°C) for one week. Food and water were available ad libitum. Rats were deprived of food for 18 h before the experiments but had access to water up to the time when gastric emptying was measured. All the experiments were performed between 12 p.m. and 4 p.m.

5.2.2. Measurement of gastric emptying.

Measurement of gastric emptying was performed as described previously (Sütő et al., 1994b). Briefly, 1.5 ml of a solution containing 1.5% methylcellulose (Sigma Chemical Co., St. Louis, MO) and 0.05% phenol red (Sigma Chemical Co., St. Louis, MO) was given intragastrically through oral intubation with a stainless steel tube to conscious rats. Rats were killed by inhalation of carbon dioxide 20 min later. In each experiment, a rat was killed immediately after intragastric administration of the test solution. Stomachs were clamped at the pylorus and cardia ends, removed, and rinsed in 0.9% saline. Stomachs were then placed in 100 ml of 0.1 N NaOH, and homogenized for 30 s. The suspension was allowed to settle for 60 min at room temperature, and 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid. After centrifugation (2.800 rpm, for 20 min) 4 ml of 0.5 N NaOH was added to the supernatant. The absorbance of the samples was read at a wavelength of 560 nm by spectrophotometer (Shimadzu UV-260). The gastric emptying for each rat was calculated according to the following formula:

gastric emptying (%) = (1 -absorbance of test sample/ absorbance of baseline) x 100.

5.2.3. Drugs and treatments.

Injections into the jugular vein (i.v.) were performed in rats under short (2.5 min) enflurane anaesthesia. The volumes of i.v. or intraperitoneal (i.p.) injections were 0.15 ml/rat or 2 ml/kg, respectively.

LPS was injected i.p. at various doses (7.5, 15 or 75 μg/kg). Rats were given either the CGRP antagonist $hCGRP_{8-37}$ (30 μg/kg, iv.) or vehicle immediately before endotoxin.

Rats were pretreated with capsaicin (25-50-50 mg/kg, s.c. over 36 h) or vehicle. The ablation of capsaicin sensitive primary sensory afferents was confirmed by the lack of eye wiping in response to corneal application of 1% NH_4Cl. 10-14 days later rats were injected with $hCGRP_{8-37}$ (30 μg/kg, i.v.) and LPS (7.5 μg/kg, i.p.) or with the respective vehicles.

5.3. Results.

The gastric emptying in vehicle-treated rats was 58.3 ± 2.5%. LPS dose dependently decreased gastric emptying. $hCGRP_{8-37}$ partially reversed the inhibition of gastric emptying delayed by LPS at 7.5 μg/kg (25.5% ± 3.1% vs. 43.1% ± 2.3%, $P<0.05$), but not at doses of 15 and 75 μg/kg (10.2% ± 1.0% vs. 14.2% ± 2.6%, 8.8% ± 21.8% vs. 11.5% ± 2.2%) (Fig. 5.1.).

Capsaicin ablation of primary sensory afferents did not influence basal gastric emptying (57.4% ± 2.8% vs. 59.7% ± 3.9%) or LPS-delayed gastric emptying (25.5% ± 3.1% vs. 30.1 ± 3.1%). In capsaicinized rats (devoid of corneal chemosensory response), $hCGRP_{8-37}$ still partially reversed gastric emptying inhibited by

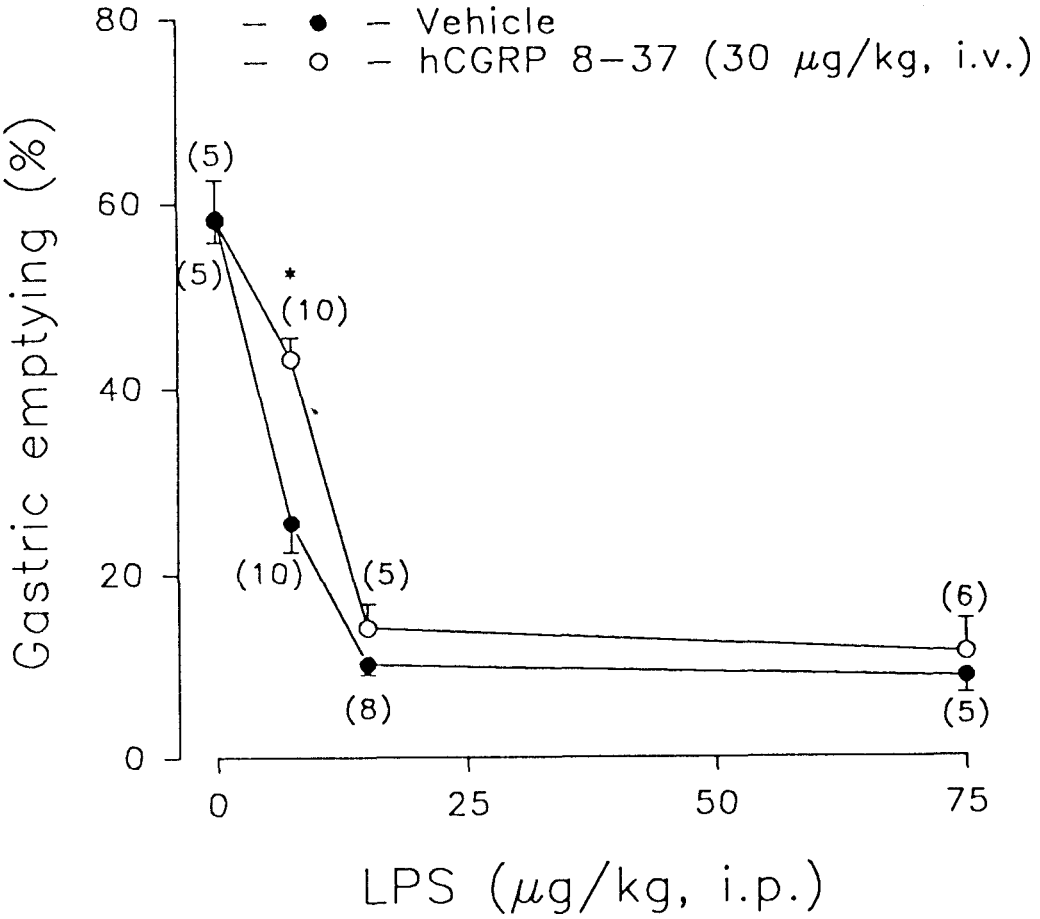

Fig. 5.1. Inhibition of i.p. LPS-induced delay of gastric emptying by i.v. injection of $hCGRP_{8-37}$ in conscious rats. $hCGRP_{8-37}$ or vehicle was injected i.v. immediately before i.p. LPS or vehicle under short enflurane anesthesia and 30 min later, the 20 min rate of gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= $P<0.05$ compared with respective vehicle-treated group.

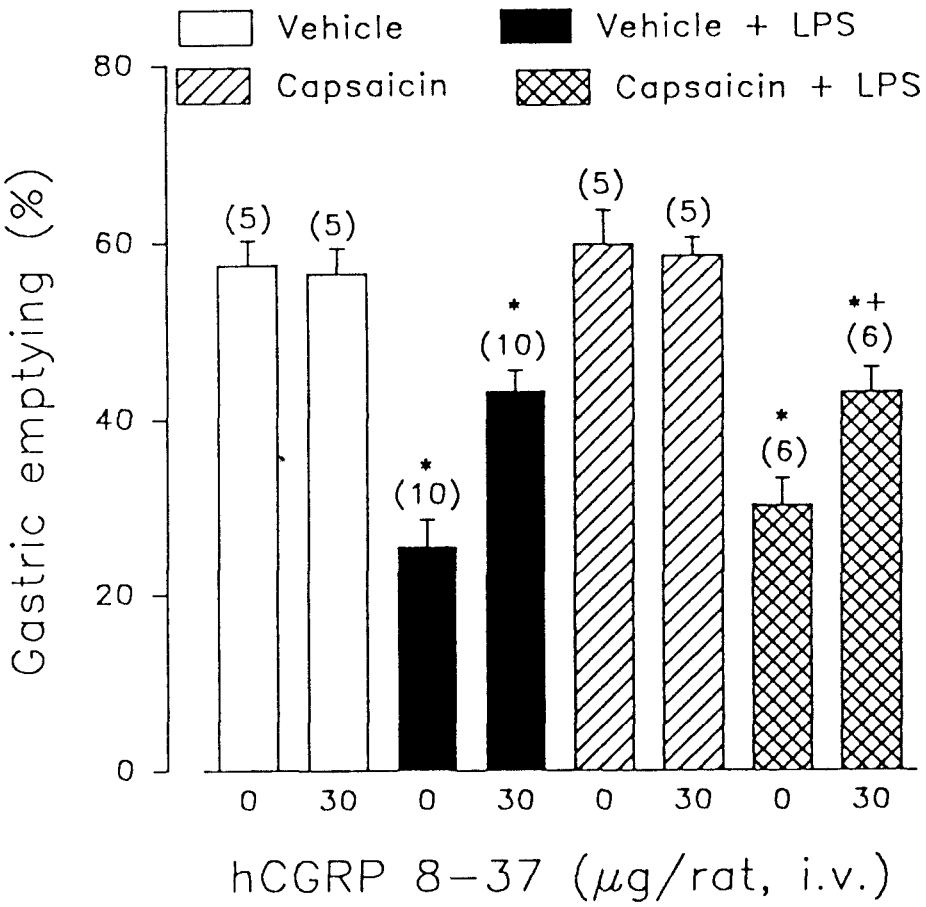

Fig 5.2. Inhibition of i.p. LPS-induced delay of gastric emptying by i.v. injection of $hCGRP_{8-37}$ in conscious rats pretreated with capsaicin (25-50-50 mg/kg) 2 weeks before the experiment. $hCGRP_{8-37}$ or vehicle was injected i.v. immediately before i.p. LPS or vehicle under short enflurane anesthesia and 30 min later, the 20 min rate of gastric emptying was measured. Each column represents the mean ± SEM of number of rats indicated in parentheses. *= P<0.05 compared with respective vehicle-treated group, += compred with respective capsaicin and LPS treated group.

LPS (30.1% ± 3.1% vs. 42.9% ± 2.8%, $P<0.05$) (Fig. 5.2.). $hCGRP_{8-37}$ did not influence gastric emptying of vehicle or capsaicin pretreated vehicle injected rats (56.5% ± 2.9% vs. 58.4 ± 2.1%).

5.4. Discussion.

LPS, a potent inducer of acute phase reaction in experimental animals dose dependently inhibits gastric emptying (Chapter 4.). Although the CGRP receptor antagonist, $hCGRP_{8-37}$ did not influence the action of the submaximal dose of CGRP, the ED_{50} dose of CGRP was partially inhibited by the antagonist. In a previous study the similar dose of $hCGRP_{8-37}$ was established to fully reverse the inhibition of gastric emptying elicited by the submaximal dose of CGRP (Plourde et al., 1993a). This may implicate that the dose administered can not be supposed to be small to inhibit CGRP on gastric emptying.

LPS results in the release of an abundant amount of CGRP during endotoxaemia (Wang et al., 1991; Wang et al., 1992a; Wang et al., 1992b; Wang et al., 1995). Recent evidence has shown that LPS elevated plasma CGRP within 30 min after its i.v. injection and enhanced CGRP mRNA level of dorsal root ganglion cells 2.5 h later (Tang et al., 1997). CGRP injected i.v. potently inhibits gastric emptying (Lenz, 1988; Plourde et al., 1993a). In the present study $hCGRP_{8-37}$ a potent $CGRP_1$ receptor antagonist (Chiba et al., 1989; Dennis et al., 1990; Donoso et al., 1990) partially antagonized the inhibition of gastric emptying induced by a dose of LPS which results in approximately 50% inhibition of gastric emptying. The gastroparesis following the i.p. injection of a maximum effective dose of LPS was unaltered by $hCGRP_{8-37}$.

αCGRP is a product of alternative processing of calcitonin gene (Rosenfeld et al., 1983) and is located primarily in the capsaicin sensitive primary afferents (Sternini, 1992). Capsaicin ablation of these neurons, confirmed by eye wiping test unaltered the gastric emptying of the nonnutrient testmeal. This finding is in line with the previous findings (Coimbra et al., 1996). Although in the present study capsaicin ablation of the capsaicin sensitive primary sensory afferents did not alter the gastroparesis induced by LPS, $hCGRP_{8-37}$ was able to partially reduce the inhibition of gastric emptying by LPS. This latter finding suggests that in response to LPS a capsaicin resistant, may be a non neural source of CGRP is mobilized to inhibit gastric emptying of nonnutient testmeal. The non neural origin of CGRP is obscure. CGRP mRNA and CGRP itself was localized within cells settled in the gastric mucosa highly likely being identified as lymphocytes (Jakab et al., 1993). However a huge increase of serum CGRP was found in endotoxemia (Chiba et al., 1989; Dennis et al., 1990; Donoso et al., 1990) immune cells were not proven yet to be able to produce CGRP in a sufficient amount to inhibit gastric emptying. Since the inhibition of gastric emptying was observed 1 h after the injection of the present dose of LPS, an inducible mediator should be suggested to result in the release of CGRP. The most likely mediator of LPS is IL-1 to release CGRP, since LPS act through IL-1 and TNF (Cybulsky et al., 1988; Long et al., 1990; Ulich et al., 1990; Dunn, 1992) and IL-1 was shown to act partially through CGRP (Coimbra et al., 1996). This hypothesis is strengthened by the ability of IL-1 receptor antagonist injected i.p. to fully inhibit the gastroparesis induced by the same dose of LPS (Sütő et al., 1996, Chapter 3.). IL-1β was shown to sensitize capsaicin sensitive

primary afferents through local release of prostaglandins (Herbert et al., 1994a) and nitic oxide (Herbert et al., 1994b), but alone was unable to release any mediator form these nerve endings.

Taken together these data, LPS injected i.p. results in the inhibition of gastric emptying which is partially mediated through the release of CGRP. The origin of CGRP is located outside the nervous system since capsaicin ablation of the primary sensory afferents did not modify the action of LPS.

6. Summary and conclusions.

Activation of the body's defense mechanisms during illness or injury involves non specific mechanisms which serve to remove the damaging agents and to facilitate tissue repair (Kushner, 1982; Cybulsky et al., 1988). This process is guided by a well orchestrated coordination of the endocrine, immune and neural systems, which results in the survival of the human body. This phenomenon was originally described by Hans Selye more than 60 years ago and was summarized as the concept of stress (Selye, 1936; Szabo et al., 1990). The involvement of the gastrointestinal system was very early discovered observing the development of gastric ulcer/erosions during stress. Stress not only results in gastric mucosa damage, but alters other gastrointestinal functions such as secretion or motility. All of these pathologies produce various gastrointestinal symptoms (e.g. loss of appetite, abdominal fullness, nausea, vomiting) for patients.

The thesis was focused on the mechanisms underlying the inhibition of gastric emptying by the most proximal inflammatory mediator the immune cascade (Dinarello, 1984a; Dinarello et al., 1986; Dinarello, 1988) IL-1β and LPS (Galanos et al., 1977; Westphal et al., 1983), which is a potent stimulant of the immune system (Cybulsky et al., 1988).

6.1. Interleukin-1ß Acts in the Brain to Inhibit Gastric Emptying in Rats: Mediation Through Prostaglandin and CRF.

A multidirectional communication has been supposed among the immune, neural and endocrine system to mediate stress response.

It has been clarified that peptides, have not only their classic function (e.g. CRF to stimulate ACTH release from the anterior pituitary or IL-1β to be the primary secretory compound of monocytes) but are involved in the regulation of other biological functions (e.g. CRF is a neurotransmitter in the DMN to inhibit gastric emptying (Taché et al., 1987) or IL-1β is localized and expressed within the CNS (Giulian et al., 1986; ; Lechan et al., 1990; Higgins et al., 1991; Ban et al., 1992).

The gastric emptying was measured by a non caloric testmeal (1.5% methylcellulose containing 0.05% phenol red) during a 20 min period in rats weighing 250-300g. All of the control (untreated or vehicle treated) rats provided an emptying rate of approximately 56% of the original volume of the test solution (1.5 ml) during this time period. It was found that IL-1β had an ED_{50} dose to inhibit gastric emptying 0.1 ng/rat following i.c. and 3.0 ng/rat following i.v. injection. The ED_{50} dose for central IL-1β is 30 fold lower than the dose for i.v. route. This suggests a central site of action which is supported by the finding which showed that there are high affinity receptors localized and expressed with neurons in the brain (Farrar et al., 1987; Katsuura et al., 1988a; Lechan et al., 1990; Takao et al., 1990; Cunningham et al., 1993). There are several explanations how circulating IL-1 may reach the CNS neurons: (1.) endothelial cells expressing receptors for IL-1β (Wong et al., 1994) may synthesize messenger molecules (Hashimoto et al., 1991): arachidonate metabolites (Dejana et al., 1987), nitric oxide (Lin et al., 1996) or IL-1 itself (Warner et al., 1987), (2.) systemic IL-1β may cross the brain-blood barrier through a saturable, carrier mediated transport system (Banks et al., 1989; Banks et al., 1991a; Banks et al., 1991b; Reimers et al., 1991; Banks et al., 1993), (3.) IL-1β may penetrate the blood-brain barrier at sites where it is fenestrated for large molecules to reach CNS

neurons: at organum vasculosum laminae terminalis (Lin et al., 1996), (4.) IL-1β may have a peripheral receptor in the liver to stimulate hepatic afferents (Niijima, 1992).

The central action of IL-1β was long lasting, at least for 6 hours. It can be explained by the slow degradation of IL-1β within the central nervous system (Banks et al., 1991a; Banks et al., 1991b) and by its ability to induce its own gene expression and peptide production (Walter et al., 1989; Wang et al., 1991). Peripheral injection of IL-1β had a shorter duration of action, 1 h after its injection the inhibition of gastric emptying is hardly observed (unpublished observations). This short duration of action correlates well with the elimination of IL-1β from the plasma mostly by the kidneys (Klapproth et al., 1989).

The inhibition of gastric emptying by either i.v. or i.c. IL-1β was fully antagonized by IL-1ra (Carter et al., 1990; Eisenberg et al., 1990; Hannum et al., 1990; Arend, 1991; Dinarello et al., 1991) injected through the respective route. A partial inhibition (46%) of the delay of gastric emptying induced by i.v. IL-1β was observed after i.c. injection of IL-1ra. There are several explanations why IL-1ra injected i.c. could not fully reverse the action of i.v. IL-1β: (1.) IL-1ra may incompletely penetrate to brain sides which may be reached by i.v. IL-1β. This explanation can be supported by the finding which has shown that IL-1β injected i.c. results in a different pattern of brain c-fos expression than injected i.v.. (2.) There is a subeffective IL-1β:IL-1ra ratio (1:33), because at least 10^2 excess of IL-1ra is required to inhibit the biological effects of IL-1β (Dinarello et al., 1991; Rothwell et al., 1994). Higher doses of IL-1ra were not tested because IL-1ra may leak to the periphery in an excess of dose 100 ng. IL-1β injected i.c. at doses higher than 100 ng (Robert et al., 1991b) were shown to have peripheral action. IL-1ra and IL-β share similar sequences

(Dinarello et al., 1991) which may predispose IL-1ra to reach the circulation after i.c. injection (Robert et al., 1991b). IL-ra injected i.v. or i.c. before the vehicle of IL-1β did not influence basal gastric emptying.

A potential peripheral action directly on smooth muscles is unlikely, because IL-1β potentiated the myotropic activity of $CaCl_2$ in an isolated rat stomach strip preparation (Mugridge et al., 1991), and the relaxing effect of IL-1β on gastric fundus strips is not altered by indomethacin (Montuschi et al., 1994).

These data demonstrate that IL-1β is not involved in the regulation of gastric emptying in basal conditions, and further confirms that IL-1β exerts its action on gastric emptying through the activation of type I IL-1 receptors (Farrar et al., 1987; Cunningham et al., 1992; Cunningham et al., 1993).

CRF (Vale et al., 1981) was originally described as the stress peptide because it stimulates the release of ACTH which in turn stimulates the adrenal cortex to secrete cortisol and corticosterone. CRF is localized in peripheral tissues such as T and B lymphocytes (Stephanou et al., 1990), testes (Dufau et al., 1993), trophoblastic and decidual tissue (Petraglia et al., 1990), pancreas (Petrusz et al., 1983), different types of cancers (Suda et al., 1984a), the human gastrointestinal tract (Kawahito et al., 1994; Kruseman et al., 1982) and in capsaicin sensitive primary sensory afferents (Skofitsch et al., 1984) as well. CRF is the peptide which is well established to modulate gastrointestinal motility during stress. Exogenous CRF injected into the CNS or the activation of endogenous CRF containing neurons inhibits the gastric emptying of a noncaloric testmeal and stimulates colonic motility (Buéno et al., 1988; Lenz et al., 1988b; Jiménez et al., 1990; Sheldon et al., 1990; Taché et al., 1993; Gue et al., 1994). Brain CRF utilizes autonomic pathways to delay gastric emptying (Taché et al., 1993). However CRF

injected i.v. was demonstrated to retard gastric emptying of noncaloric testmeal, the mechanism of action of peripheral CRF is still obscure (Taché et al., 1987; Sheldon et al., 1990; Barquist et al., 1992). IL-1β injected both i.v. or i.c./i.c.v. stimulates CRF containing CNS neurons mostly localized within the PVN of the hypothalamus (Sapolsky et al., 1987; Bernardini et al., 1990a; Bernardini et al., 1990b; Saphier et al., 1990; Ju et al., 1991; Navarra et al., 1991; Watanabe et al., 1991). There are several line of evidence that the release of CRF from CRF containing neurons after IL-1β stimulation is mediated by prostaglandins (Katsuura et al., 1985; Bernardini et al., 1990a; Bernardini et al., 1990b; Katsuura et al., 1990, Navarra et al., 1991;). The recently developed, potent CRF antagonist ([DPhe^{12}, Nle^{21,38}, C$^{\alpha}$MeLeu^{37}] CRF$_{12-41}$) injected i.c. but not i.v. fully antagonized the gastroparesis induced by i.v. IL-1β and was partially effective (52%) before i.c. injection of IL-1β. Likewise, i.c.v. IL-1β-induced stimulation of colonic motor function was shown to be partially mediated by brain CRF pathways (Fargeas et al., 1993). A similar inhibition (50%) of gastric emptying which was produced by i.c. IL-1β was achieved by i.c. CRF (600 ng/rat), and this inhibition of gastric emptying was prevented by i.c. CRF antagonist (20 μg/rat). This result leads to the conclusion that the dose of CRF antagonist was not ineffective to antagonize IL-1β mediated 50% inhibition of gastric emptying. The CRF antagonist might have been unable to penetrate to sites where CRF was released after IL-1β challenge or some other mechanisms are involved in the gastroparesis induced by ic. IL-1β. This hypothesis is supported by the fact that indomethacin was able to fully reverse the inhibition of gastric emptying induced by either i.c. or i.v. injection of IL-1β.

There are many conflicting data -due to differences of

species examined, type of PG or its analog, route of administration- regarding the role of PGs in the regulation of gastric emptying. In general, PGs retard the emptying of solid foods and mostly stimulate the emptying of liquids from the stomach (Sanders, 1984; Dubois et al., 1987; Penston et al., 1989; Nishiyama et al., 1992; Stein et al., 1994). IL-1β has the ability to release prostaglandin from several cell types including astrocytes and modulate the activity of brain neurons through prostaglandin dependent pathways (Fontana et al., 1982; Hartung et al., 1989; Nakashima et al., 1989). Central IL-1β-induced alterations of thermoregulation and hormone secretion have been ascribed to brain prostaglandin release (Bernardini et al., 1990a; Bernardini et al., 1990b; Watanabe et al., 1990; Navarra et al., 1991; Luheshi et al., 1993). Indomethacin injected i.p. reversed the centrally mediated action of IL-1 to inhibit gastric acid secretion and ulcer formation (Nakashima et al., 1989; Uehara et al., 1989; Robert et al., 1991b; Saperas et al., 1992b) and the suppression of postprandial pattern of intestinal motility (Fargeas et al., 1993). However, the sites and mechanisms through which PGs mediate i.c. IL-1β-induced inhibition of gastric emptying is obscure. IL-1β injected i.v. stimulates gastric mucosa prostaglandin formation (Robert et al., 1991a), and PGs stimulate gastric emptying (Sanders, 1984; Dubois et al., 1987; Penston et al., 1989; Nishiyama et al., 1992; Stein et al., 1994). These data argue against the peripheral action of indomethacin to reverse the inhibition of gastric emptying-induced by i.v. IL-1β. Indomethacin rather targets the central nervous system most likely to prevent IL-1β to stimulate CRF release from CNS sites. In vivo and in vitro experiments have shown that IL-1β activates hypothalamic CRF neurons in the PVN (Saphier et al., 1990; Ju et al., 1991; Watanabe et al., 1991; Chover-Gonzalez et al., 1993; Ericsson et al., 1994; Ericsson et

al., 1997), and endogenously released CRF in the brain is well established to inhibit gastric motor function in the rat (Taché et al., 1993). The present data and available neuroanatomical evidence are consistent with a possible involvement of PGs and CRF in brain mediated action of i.v. IL-1β-induced delay of gastric emptying. Most likely CRF release is influenced by PGs, because it was shown that indomethacin did not influence i.c. CRF-induced delay of gastric emptying (Sütő et al., 1994b, Chapter 2.). Recently a PG dependent activation of medullary ascending catecholaminerg neurons was shown by IL-1β (Ericsson et al., 1997), which may be responsible for the c-fos activation of CRF positive neurons in the PVN. Furthermore indomethacin did not influence basal gastric emptying, which shows that PGs are not the regulators of gastric emptying in normal rats. These findings support the notion that i.v. or i.c. IL-1β delays gastric emptying through the activation of PG and CRF dependent pathways within the CNS.

IL-1β has a CNS site of action to release PGs, and CRF through the activation of type I receptors to inhibit gastric emptying. The release of CRF may be partially mediated by PGs after i.c. or fully after i.v. injection of IL-1β.

6.2. LPS involves a peripheral release of IL-1 and CRF to delay gastric emptying.

LPS, the lipopolysaccharide component of the outer layer of gram negative bacteria (Galanos et al., 1977) is a potent inducer of the immune system to defend against infection.

LPS injection into experimental animals produces local inflammation, and entering the circulation results in profound

systemic effects such as fever (Saper et al., 1994), acute phase reaction (Kushner, 1982; van Gool et al., 1990), or in the most severe cases septic shock or disseminated intravascular coagulation (Cybulsky et al., 1988). LPS influences different gastrointestinal functions: inhibits gastric acid secretion (Saperas et al., 1994), disrupts gastrointestinal mucosal integrity (Whittle et al., 1987; Pique et al., 1988) and gastrointestinal motility (Esplugues et al., 1989; Pons et al., 1989), alters colonic water and electrolyte transport (Ciancio et al., 1992). LPS was shown to act through the stimulation of the synthesis of further mediators, including IL-1β and TNFα (Sirko et al., 1989; Zuckerman et al., 1989; Ulich et al., 1990; Foster et al., 1993). LPS was shown to induce hypothalamic (Hillhouse et al., 1993) and pituitary (Koenig et al., 1990) IL-1β.

LPS and IL-1β share many biological actions. LPS at low doses was demonstrated to activate CRF containing CNS pathways through IL-1β to stimulate the hypothalamic-pituitary-adrenal axis (Elenkov et al., 1992a; Tilders et al., 1994). Peripheral injection of endotoxin, as it was shown by c-fos immunochemistry results in the activation of neurons within the CNS which are involved in the regulation of different autonomic, endocrine and behavioral responses during inflammation (Elmquist et al., 1993; Wan et al., 1993). The injection of IL-1β produces a similar expression of c-fos suggesting that the neuronal activation elicited by peripheral LPS injection may be mediated by IL-1β (Rivest et al., 1992; Elmquist et al., 1993; Brady et al., 1994; Ericsson et al., 1994; Ericsson et al., 1997). IL-1β is well known to modulate various gastric functions including the inhibition of gastric acid secretion (Uehara et al., 1987; Saperas et al., 1990; Uehara et al., 1990) and motility (Robert

et al., 1991a; Robert et al., 1991b; McCarthy et al., 1992; van Miert et al., 1992). This action of IL-1β is mediated by CNS release of CRF and PGs through the activation of type I IL-1 receptors (Sütő et al., 1994b; Sütő et al., 1996, Chapters 2., 3.).

Interleukin receptor type I antagonist (Carter et al., 1990; Eisenberg et al., 1990; Hannum et al., 1990; Arend, 1991; Dinarello et al., 1991), a specific CRF receptor antagonist (Hernandez et al., 1993) and indomethacin were used to study the role of type I interleukin-1 receptors, CRF or PGs in the inhibition of gastric emptying by LPS. The present data show that peripheral administration of IL-1ra antagonist prevented the gastroparesis elicited by i.p. administration of LPS. The central route of administration of IL-1ra was ineffective. Increasing the dose of IL-1ra a partial inhibition was observed which may rather be ascribed for the systemic appearance of IL-1ra. Since IL-ra and IL-1β share a similar homology of amino acid sequence (Dinarello et al., 1991) and IL-1β injected i.c. at the doses superior to 100 ng was found to leak to the periphery (Wallace et al., 1992), IL-1ra at doses higher than 100 ng may be available for peripheral targets. The ineffectivity of i.c. IL-ra to inhibit LPS-induced gastroparesis may be due to several mechanisms: (1.) Neurons, other than CRF containing ones are activated by LPS or IL-1β. Both IL-1β (i.v.) and LPS (i.p.) were shown to activate oxytocin and vasopressin (Mouri et al., 1993) immunoreactivity positive neurons within the CNS. Oxytocin is an inhibitory neuropeptide of gastric emptying within the central nervous system (Sütő et al., 1994a). (2.) In response to LPS, other mediators such as IL-6 or TNFα are produced (Cybulsky et al., 1988; Long et al., 1990; Ulich et al., 1990; Dunn, 1992; Foster et al., 1993).

It is well established that CRF injected both centrally and peripherally inhibits gastric emptying (Taché et al., 1987; Sheldon et al., 1990; Barquist et al., 1992). CRF was originally isolated as the hypothalamic peptide which stimulates the secretion of corticotropin and β endorphin (Vale et al., 1981), but it was also localized in different peripheral organs/tissues including peripheral T and B lymphocytes (Stephanou et al., 1990), testes (Dufau et al., 1993), trophoblastic and decidual tissue (Petraglia et al., 1990), pancreas (Petrusz et al., 1983), different types of cancers (Suda et al., 1984a), the gastrointestinal tract (Kawahito et al., 1994; Kruseman et al., 1982) or capsaicin sensitive primary afferent neurons (Skofitsch et al., 1984; Kamilaris et al., 1992). The peripheral source of CRF and the site of action is still obscure in the inhibition of gastric emptying by i.p. LPS.

The CRF receptor antagonist was injected i.c. or i.v. at the dose which established to prevent the 50% inhibition of gastric emptying induced by CRF or IL-1β (Sütő et al., 1994b; Sütő et al., 1994b, Chapters 2., 3.). However, the i.c. injection of CRF anatgonist was not able to influence LPS-induced gastroparesis, i.v. administration of CRF antagonist prevented LPS-induced delay of gastric emptying. CRF injected i.v. was demonstrated to delay gastric emptying of a noncaloric testmeal, but this finding shows that endogenous CRF released by LPS mediates gastroparesis induced by i.p. LPS injection.

Since LPS activates the afferent limb of the immune system mediated defense against infections or damage, it is likely that the activated PBMCs may release different mediators including CRF or IL-1 to interact and result in the net activation of the immune response. To support this hypothesis interactions between peripheral CRF and cytokines originating from PBMCs were found:

(1.) However it was not confirmed in further studies (Sobel, 1990; Woudenberg et al., 1992), IL-1 was shown to mediate the effect of CRF and AVP on β-endorphin production by human PBMC (Kavelaars et al., 1989). (2.) CRF+AVP or IL-1α/β were shown to stimulate the production of ACTH-like proteins in human mononuclear cells (Reder, 1992). (3.) A further study elucidated that the presence of endotoxin determines the ability of PBMCs to synthesize IL-1 or TNF in response to CRF (Singh et al., 1990; Leu et al., 1992; Pereda et al., 1995).

These data do not allow to draw the final conclusion of the target of IL-1 and CRF released upon LPS injection to delay gastric emptying of a noncaloric testmeal. Indomethacin was not able to influence the gastric emptying delayed by LPS. This finding also argues against the central IL-1β mediation, since it was sensitive to the blockade of PG synthesis (Sütő et al., 1994b; Sütő et al., 1994b, Chapters 2., 3.). Furthermore the central injection of the receptor antagonists did not influence the action of LPS. But the role of CNS can not be excluded, since subdiaphragmatic vagotomy blocks LPS induced hyperthermia (Watkins et al., 1995), the transection of the hepatic branches of the vagus nerve inhibits illness-induced hyperalgesia (Watkins et al., 1994) and infusion of IL-1 into the portal vein results in increased firing of hepatic branches of the vagus nerve (Niijima, 1992). The possible receptor sites for IL-1β are most likely localized in the hepatic branch of the vagus nerve. Macrophages located within the liver are the primary sites to detect foreign harmful compounds and respond with the release of cytokines and other inflammatory compounds which are able to stimulate directly or indirectly the sensory nerves of the vagus (Watkins et al., 1994). This hypothesis needs further investigations.

Based on these data LPS delays the gastric emptying of a noncaloric testmeal through the peripheral release of CRF and IL-1 which is not sensitive to blocking PG synthesis. The release of these mediators may a be part of the activation of the afferent limb of the immune system.

6.3. A non-neural source of calcitonin gene related peptide contributes to the inhibition of gastric emptying by intraperitoneal endotoxin in rats.

Capsaicin sensitive primary sensory afferents are involved in the postprandial regulation of the upper gastrointestinal tract (Holzer et al., 1994; Raybould et al., 1994; Zittel et al., 1994) and in ileus due to surgery and/or peritoneal irritation (Holzer et al., 1986; Holzer et al., 1992; Plourde et al., 1993b; Takeuchi et al., 1996) in rats. αCGP is abundantly localized within the gastrointestinal tract stored mostly in primary sensory afferent fibres arising from the dorsal root ganglia, and a smaller amount was localized to in fibres originating from nodose ganglia (Sternini, 1992). A non neural source for CGRP was also described, since gastric mucosal lymphocytes produce CGRP mRNA as well (Jakab et al., 1993). CGRP injected intravenously potently inhibits gastric acid secretion, motility and the development of mucosal lesions (Maggi et al., 1987; Taché et al., 1991; Taché, 1992a). This effect of CGRP is mediated by its receptor type I and can be antagonized by the 8-37 fragment of human CGRP ($hCGRP_{8-37}$) (Chiba et al., 1989; Dennis et al., 1990; Donoso et al., 1990; Plourde et al., 1993a).

The CGRP type I receptor antagonist $hCGRP_{8-37}$ increased the

gastric emptying rate by 65% in the rats treated with the ED_{50} dose of LPS. When LPS was injected at a submaximal dose $hCGRP_{8-37}$ did not influence the gastric emptying delayed by LPS. These data allows to draw the conclusion that endogenous CGRP released in response to LPS administration may at least partially mediate the gastroparesis induced by LPS. The Ed_{50} dose of LPS might represent a lower level of stimulation of the immune system, which may be influenced by $hCGRP_{8-37}$. Higher doses of LPS may result in a robust production/release of mediators which can be hardly antagonized by the given dose of $hCGRP_{8-37}$, however this dose was established previously of antagonize the submaximal inhibition of gastric emptying by CGRP (Plourde et al., 1993a). CGRP is not involved in the regulation of gastric emptying in intact rats since $hCGRP_{8-37}$ injected i.v. did not have any effect on gastric emptying.

Capsaicin pretreatment did not influence the gastric emptying of control animals. These data confirm the earlier findings which have shown that capsaicin sensitive primary sensory afferents are not involved in the regulation of gastric emptying during physiologic conditions (Coimbra et al., 1996). Capsaicin ablation of the capsaicin sensitive primary sensory afferents did not influence the inhibition of gastric emptying after LPS treatment, and $hCGRP_{8-37}$ still had a partial inhibition on LPS-delayed gastric emptying. Recently gastric mucosal lymphocytes were shown to express CGRP mRNA (Jakab et al., 1993). Whether these cells contribute to the increase of plasma CGRP levels following LPS treatment of experimental animals (Wang et al., 1991; Griffin et al., 1992; Wang et al., 1992a; Wang et al., 1992b; Hüttemeier et al., 1993) is not proven yet, but allows to consider peripheral lymphocytes as an alternative capsaicin insensitive source of CGRP. The inhibition of gastric emptying

was observed 1 h after (Chapter 4.) the injection of the present dose of LPS. This delay of LPS mediated gastroparesis may be ascribed to an inducible mediator, which results in the release of CGRP. The most likely candidate is IL-1 to release CGRP, since LPS act through IL-1 and TNF (Cybulsky et al., 1988; Long et al., 1990; Ulich et al., 1990; Dunn, 1992) and IL-1 was shown to act partially through CGRP (Coimbra et al., 1996). This hypothesis is strengthened by the ability of IL-1 receptor antagonist injected i.p. to fully inhibit the gastroparesis induced by the same dose of LPS (Chapter 3.). IL-1β was shown to sensitize capsaicin sensitive primary afferents through local release of prostaglandins (Leung et al., 1987) and nitric oxide (Sternini, 1992), but alone was unable to release any mediator form these nerve endings.

An another explanation for $hCGRP_{8-37}$ to inhibit the LPS-induced delay of gastric emptying is a possible interaction with adrenomedullin (Vine et al., 1996). This peptide was recently isolated from phaemochromocytoma tissue, has a potent ability to delay gastric emptying (Martinez et al., 1997), and shares common receptors with CGRP (Vine et al., 1996). This hypothesis needs further confirmation utilizing specific adrenomedullin receptors.

LPS challenge of the immune system results in the delay of gastric emptying which is mediated by CGRP acting on type I receptors. CGRP is released most likely from a non neural source.

6.4. New results.

(1.) Both **central and peripheral** injection of IL-1β induced potent inhibition of gastric emptying. The central dose of IL-1β was 33 fold lower than the peripheral dose, which supports a **central target** for IL-1β to delay the gastric emptying of a noncaloric testmeal.

(2.) IL-1β delays gastric emptying through the activation of **specific interleukin-1 receptors**. These receptors are type 1 Tcell/fibroblast receptors for IL-1.

(3.) IL-1β is not involved in the regulation of basal gastric emptying.

(4.) The gastroparesis elicited by both central and peripheral IL-1β is regulated by CNS **prostaglandins**.

(5.) Central IL-1β involves **CRF** release to delay gastric emptying.

(6.) Peripheral IL-1β is partially mediated by central release of **CRF**.

(7.) **LPS** injected intraperitoneally **dose dependently** decreased gastric emptying.

(8.) The gastroparesis induced by LPS has a **delayed onset of action** and is **long lasting**, most likely due to the synthesis and/or release of secondary mediator molecules such as IL-1β or CRF.

(9.) This is the first time to demonstrate that LPS delayed gastric emptying is mediated by endogenously released peripheral **IL-β and CRF**.

(10.) LPS-induced delay of gastric emptying is not prostaglandin dependent.

(11.) LPS-induced delay of gastric emptying is **partially mediated**

by CGRP, originated from a **nonneural**, capsaicin resistant **source**.

These data further support **interactions between the immune system and neuroregulation of gastric function** which may have implications in the understanding of gastric stasis associated with some pathology activating the immune system

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8. References.

Arend W.P. (1991): Interleukin-1 receptor antagonist a new member of the interleukin-1 family. J Clin Invest 88:1445-1451.

Audhya T., Hollander C.S., Schlesinger D.H., Hutchinson B. (1989): Structural characterization an localization of corticotropin releasing factor in testis. Biochem Biophys Acta 995:10-16.

Ban E., Haour F., Lenstra R. (1992): Brain interleukin 1 gene expression induced by peripheral lipopolysaccharide administration. Cytokine 4:48-54.

Banks W.A., Kastin A.J., Durham D.A. (1989): Bidirectional transport of interleukin-1 alpha across the blood-brain barrier. Brain Res Bull 23:433-437.

Banks W.A., Kastin A.J. (1991a): Blood to brain transport of interleukin links the immune and central nervous system. Life Sci 48:PL-117-PL-121.

Banks W.A., Ortiz L., Plotkin S.R., Kastin A.J. (1991b): Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. J Pharmacol Exp Ther 259:988-996.

Banks W.A., Kastin A.J., Gutierrez E.G. (1993): Interleukin-1 alpha in blood has direct access to cortical brain cells.

Neurosci Lett 163:41-44.

Barbanel G., Ixart G., Szafarczyk A., Malaval F., Assenmacher I. (1989): Intrahypothalamic infusion of interleukin-1 beta increases the release of corticotropin-releasing hormone (CRH-41) and adrenocorticotropic hormone (ACTH) in free-moving rats bearing a push-pull cannula in the median eminence. Brain Res 516:31-36.

Barocelli E., Impicciatore M., Seaton J., Conter R., Kauffman G. (1991): Localizatiòn of central prostaglandin E2 antisecretory effects. Gastroenterology 100:320-327.

Barquist E., Zinner M., Rivier J., Taché Y. (1992): Abdominal surgery-induced delayed gastric emptying in rats: role of CRF and sensory neurons. Am J Physiol 262:G616-G620.

Berkenbosch F., van Oers J., del Rey A., Tilders F., Besedovsky H. (1987): Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. Science 238:524-526.

Bernardini R., Calogero A.E., Mauceri G., Chrousos G.P. (1990a): Rat hypothalamic corticotropin-releasing hormone secretion in vitro is stimulated by interleukin-1 in an eicosanoid-dependent manner. Life Sci 47:1601-1607.

Bernardini R., Mauceri G., Chiarenza A. (1990b): Rat hypothalamic corticotropin-releasing hormone secretion is stimulated by interleukin-1 in an eicosanoid-dependent manner. Pharm Res 22(Suppl3):61-68.

Brady L.S., Lynn A.B., Herkenham M., Gottesfeld Z. (1994): Systemic interleukin-1 induces early and late patterns of c-fos mRNA expression in brain. J Neurosci 14:4951-4964.

Breder C.D., Dinarello C.A., Saper C.B. (1988): Interleukin-1 immunoreactive innervation of the human hypothalamus. Science 240:321-324.

Brynskov J., Tvede N., Andersen C.B., Vilien M. (1992): Increased concentrations of interleukin-1 beta, interleukin-2, and soluble interleukin-2 receptors in endoscopical mucosal biopsy specimens with acitve inflammatory bowel disease. Gut 33:55-58.

Buéno L., Gué M. (1988): Evidence for the involvement of corticotropin-releasing factor in the gastrointestinal disturbances induced by acoustic and cold stress in mice. Brain Res 441:1-4.

Cabanis P.J.G. (1802): Introduction. In
Mora G. (ed.): On the Relation Between the Physical and Moral Aspects of Man. Baltimore MD: John Hopkins University Press.
Cannon J., Dinarello C.A. (1985): Increased plasma interleukin-1 activity in women after ovulation. Science 227:1247-1249.

Cannon J.G., Kluger M.J. (1983): Endogenous pyrogen activity in human plasma after exercise. Science 220:617-619.

Cannon W.B. (1902): The movements of the intestines studied by means of the Roentgen rays. Am J Physiol 6:251-277.

Carter D.B., Deibel M.R.,Jr., Dunn C.J., Tomich C.-J.C., Laborde A.L., Slightom J.L., Berger A.E., Bienkowski M.J., Sun F.F., Mc Ewan R.N., Harris P.K.W., Yem A.V., Waszak G.A., Chosay J.G., Sieu L.C., Hardee M.M., Zurcher-Neely H.A., Reardon I.M., Heinrikson R.L., Truesdell S.E., Shelly J.A., Eessalu T.E., Taylor B.M., Tracey D.E. (1990): Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. Nature 344:633-638.

Chan R.K.W., Brown E.R., Ericsson A., Kovacs K.J., Sawchenko P.E. (1993): A comparison of two immediate-early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine circuitry. J Neurosci 13:5126-5138.

Chiba T., Yamaguchi A., Yamatani T., Nakamura A., Morishita T., Inui T., Fukase M., Noda T., Fujita T. (1989): Calcitonin gene-related peptide receptor antagonist human CGRP $_{8-37}$. Am J Physiol 256:E331-E335.

Chover-Gonzalez A.J., Harbuz M.S., Lightman S.L. (1993): Effect of adrenalectomy and stress on interleukin-1 beta-mediated activation of hypothalamic corticotropin-releasing factor mRNA. J Neuroimmunol 42:155-160.

Ciancio M.J., Vitiritti L., Dhar A., Chang E.B. (1992): Endotoxin-induced alterations in rat colonic water and electrolyte transport. Gastroenterology 103:1437-1443.

Coceani F., Lees J., Bishai I. (1988): Further evidence

implicating prostaglandin E2 in the genesis of pyrogen fever. Am J Physiol 254:R463-R469.

Coimbra C.R., Plourde V. (1996): Abdominal surgery-induced inhibition of gastric emptying is mediated in part by interleukin-1 beta. Am J Physiol 270:R556-R560.

Cullen J.J., Caropreso D.K., Ephgrave K.S. (1995): Effect of endotoxin on canine gastrointestinal motility and transit. J Surg Res 58:90-95.

Cunningham E.T.,Jr., Wada E., Carter D.B., Tracey D.E., Battey J.F., De Souza E.B. (1992): In situ histochemical localization of type I interleukin-I receptor messenger RNA in the central nervous system, pituitary, and adrenal gland of the mouse. J Neurosci 12:1101-1114.

Cunningham E.T.,Jr., De Souza E.B. (1993): Interleukin 1 receptors in the brain and endocrine tissues. Immunol Today 14:171-176.

Cybulsky M.I., Chan M.K.W., Movat H.Z. (1988): Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor necrosis factor and their implication in gram-negative infection. Lab Invest 58:365-378.

Dejana E., Breviario F., Erroi A., Bussolino F., Mussoni L., Gramse M., Pintucci G., Casali B., Dinrello C.A., VanDamme J., Mantovani A. (1987): Modulation of endothelial cell functions by different molecular species of interleukin-1. Blood 69:695-699.

Dennis T., Fournier A., Cadieux A., Pomerleau F., Jolicoeur F.B., St.Pierre S., Quirion R. (1990): . J Pharmacol Exp Ther 254:123-127.

Derijk R., van Rooijen N., Tilders F.J.H., Besedovsky H.O., del Rey A., Berkenbosch F. (1991): Selective depletion of macrophages prevents pituitar-adenal activation in response to subpyrogenic, but not to pyrogenic, doses of bacterial endotoxin in rats. Endocrinology 129:330-338.

Derijk R., Berkenbosch F. (1992): Development and application of a radioimmunoassay to detect interleukin-1 in rat peripheral circulation. Am J Physiol 263:E1092-E1098.

Dinarello C.A. (1984a): Interleukin-1 and the pathogenesis of acute-phase response. N Engl J Med 311:1413-1418.

Dinarello C.A., Clowes G.H.A.,Jr., Gordon A.H., Saravis C.A., Wolff S.M. (1984b): Cleavage of human interleukin 1: Isolation of a peptide fragment from plasma of febrile humans and activated monocytes. J Immunol 133:1332-1338.

Dinarello C.A., Cannon J.G., Mier J.W., Bernheim HA., LoPreste G., Lynn D.L., Love R.N., Webb A.C., Auron P.E., Reuben R.C., Rich A., Wolff S.M., Putney S.D.(1986): Multiple biological activites of human recombinant interleukin 1. J Clin Invest 66:1734-1739.

Dinarello C.A. (1988): Biology of interleukin 1. FASEB J

2:108-115.

Dinarello C.A., Thompson R.C. (1991): Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. Immunol Today 12:404-410.

Donoso M.V., Fournier A., St-Pierre S., Huidobro-Toro J.P. (1990): Pharmacological characterization of CGRP 1 receptor subtype in the vascular system of the rat: Studies with hCGRP fragments and analogs. Peptides 11:885-889,

Dubois A., Conklin J.J. (1987): Prostaglandins and gastric emptying. Adv Prostaglandin Thromboxane Leukot Res 17A:370-372.

Dufau M.L., Tinajero J.C., Fabbri A. (1993): Corticotropin-releasing factor: An antireproductive hormone of the testis. FASEB J 7:299-307.

Dunn A.J. (1992): The role of interleukin-1 and tumor necrosis factor alpha in the neurochemical and neuroendocrine responses to endotoxin. Brain Res Bull 29:807-812.

Ebisui O., Fukata J., Tominaga T., Murakami N., Kobayashi H., Segawa H., Muro S., Naito Y., Nakai Y., Masiu Y., Nishida T., Imura H. (1992): Roles of interleukin-1 alpha and -1 beta in endotoxin-induced suppression of plasma gonadotropin levels in rats. Endocrinology 130:3307-3313.

Eisenberg S.P., Evans R.J., Arend W.P., Verderber E., Brewer M.T., Hannum C.H., Thompson R.C. (1990): Primary structure and

functional expression from coplementary DNA of a human interleukin-1 receptor antagonist. Nature 343:341-346.

Elenkov I.J., Kiss J., Stark E., Bertok L. (1992a): CRF-dependent and CRF-independent mechanisms involved in hypophysial-adrenal system activation by bacterial endotoxin. Acta Physiol Hung 79:355-363.

Elenkov I.J., Kovacs K., Kiss J., Bertok L., Vizi E.S. (1992b): Lipopolysaccharide is able to baypass corticotropin-releasing factor in affecting plasma ACTH and corticosterone levels: evidence from rats with lesions of the paraventricular nucleus. J Endocrinol 133:231-236.

Elmquist J.K., Ackerman M.R., Register K.B., Rimler R.B., Ross L.R., Jacobson C.D. (1993): Induction of Fos-like immunoreactivity in the rat brain following Pasturella multocida endotoxin A administration. Endocrinology 133:3054-3057.

Ericsson A., Kovacs K.J., Sawchenko P.E. (1994): A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. J Neurosci 14:897-913.

Ericsson A., Arias C., Sawchenko P.E. (1997): Evidence for an intramedullary prostaglandin-dependent mechanism in the activation of stress-related neuroendocrine circuitry by intravenous interleukin-1. J Neurosci 17:7166-7179.

Esplugues J.V., Whittle B.J.R. (1989): Mechanisms contributing

to gastric motility changes induced by PAF-acether and endotoxin in rats. Am J Physiol 256:G275-G282.

Fabry Z., Fitzsimmons K.M., Herlain J.A., Moninger T.O., Dobbs M.B., Hart M.N. (1993): Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes. J Neuroimmunol 47:23-34.

Fargeas M.-J., Fioramonti J., Buéno L. (1993): Central action of interleukin-1 beta on intestinal motility in rats: Mediation by two mechanisms. Gastroenterology 104:377-383.

Farrar W.L., Kilian P.L., Ruff M.R., Hill J.M., Pert C.B. (1987): Visualization and characterization of interleukin 1 receptors in brain. J Immunol 139:459-463.

Fontana A., Kristensen F., Dubs R., Gemsa D., Weber E. (1982): Production of prostaglandin E and interleukin-1 like factor by cultured astrocytes and C6 glioma cells. J Immunol 129:2413-2419.

Foster S.J., McCormick L.M., Ntolosi B.A., Campbell D. (1993): Production of TNF aplha by LPS-stimulated murine, rat and human blood and its pharmacological modulation. Agents and Actions 38:C77-C79.

Galanos C.O., Luderitz O., Rietschel E.T., Westphal O. (1977): Newer aspects of the chemistry and biology of bacterial lipopolysaccharides, with special refrence to their lipid A component. In Goodwin T.W. (ed.): Biochemstry of Lipids II.

Baltimore MD: University Park Press, 1977, p. 239-247

Gaykema R.P., Dijkstra I., Tilders F.J. (1995): Supdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. Endocrinology 136:4717-4720.

Giulian D., Baker T.J., Shih L.N., Lachman L.B. (1986): Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 164:594-604.

Griffin E.C., Aiyar N., Slivjak M.J., Smith E.F. (1992): Effect of endotoxicosis on plasma and tissue levels of calcitonin gene-related peptide. Circ Shock 38:50-54.

Gue M., Gleizes-Escala C., Del Rio-Lacheze C., Junien J.-L., Bueno L. (1994): Reversal of CRF- and dopamine-induced stimulation of colonic motility by CCK and igmesine (JO 1784) in the rat. Br J Pharmacol 111:930-934.

Hamilton A.J., Carr D.B., LaRovere J.M., Black P.ML. (1986): Endotoxic shock elicits greater endorphin secretion than hemorrhage. Circ Shock 19:47-54.

Hannum C.H., Wilcox C.J., Arend W.P., Joslin F.G., Dripps D.J., Hemidal P.L., Armes L.G., Sommer A., Eisenber S.P., Thompson R.C. (1990): Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 343:336-340.

Hartung H.-P., Schafer B., Heininger K., Toyka K.V. (1989):

Recombinant interleukin-1 beta stimulates eicosanoid production in rat primary culture astrocytes. Brain Res 489:113-119.

Hashimoto M., Ishikawa Y., Yokota S., Goto F., Bando T., Sakakibara Y., Iriki M. (1991): Action site of circulating interleukin-1 on the rabbit brain. Brain Res 540:217-223.

Herbert M.K., Holzer P. (1994a): Interleukin-1 beta enhances capsaicin-induced neurogenic vasodilatation in the rat skin. Br J Pharmacol 111:681-686.

Herbert M.K., Holzer P. (1994b): Nitric oxide mediates the amplification by interleukin-1 beta of neurogenic vasodilatation in the rat skin. Eur J Pharmacol 260:89-93.

Hernandez J.F., Kornreich W., Rivier C., Miranda A., Yamamoto G., Andrews J., Taché Y., Vale W., Rivier J. (1993): Synthesis and relative potency of new constrained CRF antagonists. J Med Chem 36:2860-2867.

Hetier E., Ayala J., Denefle P., Bousseau A., Rouget P., Mallat M., Prochiantz A.(1988): Brain macrophages synthesize interleukin-1 and interleukin-1 mRNAs in vitro. J Neurosci Res 21:391-397.

Higgins G.A., Olschowka J.A. (1991): Induction of interleukin-1 beta mRNA in adult rat brain. Molecular Brain Research 9:143-148.

Hillhouse E.W., Mosley K. (1993): Peripheral endotoxin induces

hypothalamic immunoreactive interlukin-1 beta in the rat. Br J Pharmac 109:289-290.

Holzer H.H., Turkelson C.M., Solomon T.E., Raybould H.E. (1994): Intestinal lipid inhibits gastric emptying via CCK and vagal capsaicin-sensitive afferent pathway in rats. Am J Physiol 267:G625-G629.

Holzer P., Lippe I., Holzer Petsche U. (1986): Inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. Gastroenterology 91:360-363.

Holzer P., Lippe I.T., Amann R. (1992): Participation of capsaicin-sensitive afferent neurons in gastric motor inhbition caused by laparotomy and intraperitoneal acid. Neuroscience 48:715-722.

Hori T., Shibata M., Nakashima T., Yamasaki M., Asami A., Asami T., Koga H. (1988): Effects of interleukin-1 and arachidonate on the preoptic and anterior hypothalamic neurons. Brain Res Bull 20:75-82.

Howard R.L., Menzaghi F., Heinrichs S.C., Rivier J., Koob G.F. (1993): A long lasting competitive releasing factor analog antagonist without partial agonist effect. Soc Neurosci 19:2.

Hüttemeier P.C., Ritter E.F., Benveniste H. (1993): Calcitonin gene-related peptide mediates hypotension and tachycardia in endotoxic rats. Am J Physiol 265:H767-H769.

Ishikawa T., Nagata S., Ago Y., Takahashi K., Karibe M. (1990): The central inhibitory effect of interleukin-1 on gastric acid secretion. Neurosci Lett 119:114-117.

Jakab G., Webster H.F., Salamon I., Mezey E. (1993): Neural and non-neural origin of calcitonin gene-related peptide (CGRP) in the gastric mucosa. Neuropept 24:117-122.

Jiménez M., Buéno L. (1990): Inhibitory effects of neuropeptide Y (NPY) on CRF and stress-induced cecal motor response in rats. Life Sci 47:205-211.

Ju G., Zhang X., Jin B.Q., Huang C.S. (1991): Activation of corticitropin-releasing factor-containing neurons in the paraventricular nucleus of the hypothalamus by interleukin-1 beta in rat. Neurosci Lett 132:151-154.

Kamilaris T.C., Johnson E.O., Calogero A.E., Calogeras K.T., Bernardini R., Chrousos G.P., Gold P.W. (1992): Cholecystokinin-octapeptide stimulates hypothalamic-pituitary-adrenal function in rats: Role of corticotropin-releasing hormone. Endocrinology 130:1764-1774.

Katsuura G., Gottschall P.E., Dahl R.R., Arimura A. (1985): Adrenocorticotropin release induced by intacerebroventricular injection of recombinant human interleukin-1 in rats: Possible involvement of prostaglandin. Endocrinology 122:1773-1779.

Katsuura G., Gottschall P.E., Arimura A. (1988a): Identification

of high-affinity receptor for interleukin-1 beta in rat brain. Biochem Biophys Res Comm 156:61-67.

Katsuura G., Gottschall P.E., Dahl R.R., Arimura A. (1988b): Adrenocorticotropin release induced by intracerebroventricular injection of human recombinant interleukin-1 in rats: Possible involvement of prostaglandin. Endocrinology 122:1773-1779.

Katsuura G., Arimura A., Koves K., Gotschall P.E. (1990): Involvement of organum vasculosum of lamina terminalis and preoptic area in interleukin-1 beta -induced ACTH release. Am J Physiol 258:E163-E171.

Kavelaars A., Ballieux R.E., Heijnen C.J. (1989): The role of IL-1 in the corticotropin-releasing factor and arginine-vasopressin-induced secretion of immunoreactive beta-endorphin by human peripheral blood mononuclear cells. J Immunol 142:2338-2342.

Kawahito Y., Sano H., Kawata M., Yuri K., Mukai S., Yamamura Y., Kato H., Chrousos G.P., Wilder R.L., Kondo M. (1994): Local secretion of corticotropin-releasing hormone by enterochromaffin cells in human colon. Gastroenterology 106:859-865.

Klapproth J., Castell J., Geiger T., Andus T., Heinrich P. (1989): Fate and biological action of human recombinant interleukin 1 beta in the rat in vivo. Eur J Immunol 19:1485-1490.

Koenig J.I., Snow K., Clark B.D., Toni B.D., Cannon J.G., Shaw

A.R., Dinarello C.A., Reichlin S., Lee S.L., Lechan R.M. (1990): Intrinsic pituitary interleukin-1 beta is induced by bacterial lipopolysaccharide. Endocrinology 126:3053-3058.

Korneva E.A., Rybakina E.G., Fomicheva E.E., Kozinets I.A., Shkhinek E.K. (1992): Altered inteleukin-1 production in mice exposed to rotation stress. Int J Tissue React 14:219-224.

Kruseman A.C.N., Linton E.A., Lowry P.J., Rees L.H., Besser G.M. (1982): Corticotropin-releasing factor immunorectivity in human gastrointestinal tract. Lancet II:1245-1246.

Kushner I. (1982): The phenomenon of the acute phase response. Ann N Y Acad Sci 389:39-48.

La Feuvre R.A., Aisenthal L., Rothwell N.J. (1991): Involvement of corticotropin releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res 555:245-250.

Lechan R.M., Toni R., Clark B.D., Cannon J.G., Shaw A.R., Dinarello C.A., Reichlin S. (1990): Immunoreactive interleukin-1 beta localization in the rat forebrain. Brain Res 514:135-140.

Lee S.C., Liu W., Dickson D.W., Brosnan C.F., Berman J.W. (1993): Cytokine production by human fetal microglia and astrocytes. Differential production by lipopolysaccharide and IL-1 beta. J Immunol 150:2659-2667.

Lenz H.J. (1988a): Calcitonin and CGRP inhibit gastrointestinal

transit via distinct neuronal pathways. Am J Physiol 254:G920-G924.

Lenz H.J., Burlage M., Raedler A., Greten H. (1988b): Central nervous system effects of corticotropin-releasing factor on gastrointestinal transit in the rat. Gastroenterology 94:598-602.

LePard K.J., Stephens R.L.J. (1994): Serotonin inhibits gastric acid secretion through a 5-hydroxytryptamine 1-like receptor in the rat. J Pharmacol Exp Ther 270:1139-1144.

Leu S.J., Singh V.K. (1992): Stimulation of interleukin-6 production by corticotropin-releasing factor. Cell Immunol 143:220-227.

Leung F.W., Tallos E.G., Taché Y., Guth P.H. (1987): Calcitonin gene-related peptide inhibits acid secretion without modifiyng blood flow. Am J Physiol 252:G215-G218.

Lin J.H., Lin M.T. (1996): Inhibition of nitric oxide synthase or cyclo-oxygenase pathways in organum vasculosum laminae terminalis attenuates interleukin-1 beta fever in rabbits. Neurosci Lett 208:155-158.

Long N.C., Otternes I., Kunkel S.L., Vander A.J., Kluger M.J. (1990): Roles of interleukin 1 beta and tumor necrosis factor in lipopolysaccharide fever in rats. Am J Physiol 259:R724-R728.

Luheshi G., Hopkins S.J., Lefeuvre R.A., Dascombe M.J., Ghiara P., Rothwell N.J. (1993): Importance of brain IL-1 type II

receptors in fever and thermogenesis in the rat. Am J Physiol 265:E585-E591.

Maggi C.A., Evangelista S., Giuliani S., Meli A. (1987): Anti-ulcer activity of calcitonin gene-related peptide in rats. Gen Pharmacol 18:33-34.

Main C., Blennerhassett P., Collins S.M. (1993): Human recombinant interleukin-1 beta suppresses acetylcholine release from rat myenteric plexus. Gastroenterology 104:1648-1654.

Martinez V., Cuttitta F., Taché Y. (1997): Central action of adrenomedullin to inhibit gastric emptying in rats. Endocrinology 138:3749-3755.

McCarthy D.O., Daun J.M. (1992): The role of prostaglandins in interleukin-1 induced gastroparesis. Physiol Behav 52:351-353.

Minami M., Kutaishi Y., Satoh M. (1991): Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain. Biochem Biophys Res Comm 176:593-598.

Moldawer L.L., Gelin J., Schersten T., Lundholm K.G. (1987): Circulating interleukin 1 and tumor necrosis factor during inflammation. Am J Physiol 253:R922-R928.

Monnikes H., Schmidt B.G., Raybould H.E., Taché Y. (1992): CRF in the paraventricular nucleus mediates gastric and colonic motor response to restraint stress. Am J Physiol 262:G137-G143.

Montuschi P., Tringali G., Curro D., Ciabattoni G., Parente L., Preziosi P., Navarra P.(1994): Evidence that interleukin-1 beta and tumor necrosis factor inhibit gastric fundus motility via the lipoxygenase pathways. Eur J Pharmacol 252:253-259.

Montuschi P., Tringali G., Mirtella A., Parente L., Ragazzoni E., Preziosi P., Navarra P.(1996): Interleukin-1 beta release from rat gastric fundus. Am J Physiol 271:G275-G281.

Morrow N.S., Quinonez G., Weiner H., Taché Y., Garrick T. (1995): Interleukin-1 beta (IL-1 beta) microinjected into the dorsal vagal complex (DVC) inhibits TRH analogue-induced stimulation of gastric contractility in rats. Am J Physiol 269:G196-G202.

Mouri T., Itoi K., Takahashi K., Suda T., Murakami O., Yoshinaga K., Andoh N., Ohtani N., Masuda T., Sasano N. (1993): Colocalization of corticotropin-releasing factor and vasopressin in the paraventricular nucleus of the human hypothalamus. Neuroendocrinol 57:34-39.

Mugridge K.G., Perretti M., Becherucci C., Parente L. (1991): Persistent effects of interleukin-1 on smooth muscle preparations from adrenalectomized rats: Implications for increased phospholipase-A2 activity via stimulation of 5-lipoxygenase. J Pharmacol Exp Ther 256:29-37.

Nakashima T., Hori T., Mori T., Kuriyama K., Mizuno K. (1989): Recombinant human interleukin-1 beta alters the activity of preoptic thermosensitive neurons in vitro. Brain Res Bull 23:209-213.

Nava E., Palmer R.M.J., Moncada S. (1992): The role of nitric oxide in endotoxic shock: Effects of NG-monomethyl-L-arginine. J Cardiovasc Pharmacol 20(Suppl.12):S132-S134.

Navarra P., Tsagarakis S., Faria M.S., Rees L.H., Besser G.M., Grossmann A.B. (1991): Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus in vitro via the eicosanoid cyclooxygenase pathway. Endocrinology 128:37-44.

Nieto-Sampdero M., Berman M.A. (1987): Interleukin-1-like activity in rat brain: Sources, targets, and effect of injury. J Neurosci Res 17:214-219.

Niijima A. (1992): The afferent discharges from sensors for interleukin-1-beta in the hepato-portal system in the anesthetized rat. J Physiol Lond 446:236P (Abstract).

Nishiyama K., Katori M., Ueno A., Ohno T., Saigenji K. (1992): Suppression of myoelectrical activity of gastric smooth muscle by endogenous gastric prostaglandin E2. Dig Dis Sci 37:1002-1008.

Okumura T., Uehara A., Okamura K., Takasugi Y., Namiki M. (1990): Inhibition of gastric pepsin secretion by peripherally or centrally injected interleukin-1 in rats. Biochem Biophys Res Comm 167:956-961.

Okumura T., Uehara A., Kitamori S., Okamura K., Takasugi Y., Namiki M. (1991): Prevention by interleukin-1 of intracisternally

injected thyrothropin-releasing hormone (TRH)-induced gastric mucosal lesions in rats. Neurosci Lett 125:31-33.

Oppenheim J.J., Kovacs E.J., Matsushima K., Durum S.K. (1986): There is more than one interleukin 1. Immunol Today 7:45-56.

Penston J.G., Wormsley K.G. (1989): The effects of prostaglandins on gastric emptying. Scand J Gastroenterol 164:127-132.

Pereda M.P., Sauer J., Castro C.P., Finkielman S., Stalla G.K., Holsboer F., Arzt F. (1995): Corticotropin-releasing hormone differentially modulates the interleukin-1 system according to the level of monocyte activation by endotoxin. Endocrinology 136:5504-5510.

Perretti M., Mugridge K.G., Wallace J.L., Parente L. (1992): Reduction of aspirin-induced gastric damage in rats by interleukin-1 beta: Possible involvement of endogenous corticoteroids. J Pharmacol Exp Ther 261:1238-1247.

Petraglia F., Garuti G.C., De Ramundo B., Angioni S., Genazzani A.R., Bilezikjan L.M. (1990): Mechanism of action of interleukin-1 beta in increasing corticotropin-releasing factor and adrenocorticotropin hormone release from cultured human placental cells. Am J Obstet Gynecol 163:1307-1312.

Petrusz P., Merchenthaler I., Maderdrut J.L., Vigh S., Schally A.V. (1983): Corticotropin-releasing factor (CRF)-like immunoreactivity in the vertebrate endocrine pancreas. Proc Natl Acad Sci USA 80:1721-1725.

Pique J.M., Yonei Y., Whittle B.J.R., Leung F.W., Guth P.H. (1988): Indomethacin potentiates endotoxin-induced blood flow reduction and histological injury in rat gastric mucosa. Br J Pharmac 93:925-931.

Plourde V., St-Pierre S., Fournier A., Taché Y. (1993a): CGRP 8-27 blocks the inhibition of gastric emptying induced by intravenous injection of alpha-CGRP in rats. Life Sci 52:857-862.

Plourde V., Wong H.C., Walsh J.H., Raybould H.E., Taché Y. (1993b): CGRP antagonists and capsaicin on celiac ganglia partly prevent postoperative gastric ileus. Peptides 14:1225-1229.

Pons L., Droy-Lefaix M.T., Braquet P., Bueno L. (1989): Involvement of platelet-activating factor (PAF) in endotoxin-induced intestinal motor disturbances in rats. Life Sci 45:533-541.

Raybould H.E., Lloyd K.C. (1994): Integration of postprandial function in the proximal gastrointestinal tract. Role of CCK and sensory pathways. Ann N Y Acad Sci 713:143-156.

Reder A.T. (1992): Regulation of production of adrenocorticotropin-like proteins in human mononuclear cells. Immunology 77:436-442.

Reimers J., Wogensen L.D., Welinder B., Hejnaes K.R., Poulsen S.S., Nilsson P., Nerup J. (1991): The pharmacokinetics, distribution and degradation of human recombinant interleukin 1

beta in normal rats. Scand J Immunol 34:597-617.

Reimund J.M., Wittersheim C., Dumont S., Muller C.D., Kenney J.S., Baumann R., Poindron P., Duclos B. (1996): Increased production of tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. Gut 39:684-689.

Rivest S., Torres G., Rivier C. (1992): Differential effects of central and peripheral injection of interleukin-1 beta on brain c-fos expression and neuroendocrine functions. Brain Res 587:13-23.

Rivier C., Chizzonite R., Vale W. (1989a): In the mouse, the activation of the hypothalamic-pituitary adrenal axis by a lipopolysaccharide (endotoxin) is mediated through interleukin-1. Endocrinology 125:2800-2805.

Rivier C., Vale W., Brown M. (1989b): In the rat, interleukin-1alpha and -beta stimulate adrenocorticotropin and catecholamine release. Endocrinology 125:3096-3102.

Rivier C. (1990): Role of endotoxin and interleukin-1 in modulating ACTH,LH and sex steroid secretion. In Porter J.C., Jezová D. (eds.): Circulating Regulatoty Factors and Neuroendocrine Function. New York: Plenum Press, p. 295-301.

Rivier C., Vale W. (1991): Stimulatory effect of interleukin-1 on adrenocorticotropin secretion in the rat: Is it modulated by prostaglandins? Endocrinology 129:384-388.

Robert A., Olafsson A.S., Lancaster C., Zhang W. (1991a): Interleukin-1 is cytoprotective, antisecretory, stimulates PGE2 synthesis by the stomach, and retards gastric emptying. Life Sci 48:123-134.

Robert A., Saperas E., Zhang W., Olafsson A.S., Lancaster C., Tracey D.E., Chosay J.G., Taché Y. (1991b): Gastric cytoprotection by intracisternal interleukin-1 beta in the rat. Biochem Biophys Res Comm 174:1117-1124.

Rosenfeld M.G., Mermod J.-J., Amara S.G., Swanson L.W., Sawchenko P.E., Rivier J., Vale W., Evans R.M. (1983): Production of a novel neuropeptide ensoded by the calcitonin gene via tissue-specific RNA processing. Nature 304:129-135.

Rothwell N.J. (1991): Functions and mechanisms of interleukin 1 in the brain. Trends Pharmacol Sci 12:430-436.

Rothwell N.J., Luheshi G. (1994): Pharmacology of interleukin-1 actions in the brain. Adv Pharmacol 25:1-20.

Ruwart M.J., Rush B.D. (1984): The effects of PGF2 alpha, PGE2 and 16,16-dimethyl-PGE2 on gastric emptying and small intestinal transit in rat. Prostaglandins 28:915-928.

Sakagushi T., Ohtake M. (1985): Inhibition of gastric motility induced by activation of the hypothalamic paraventricular nucleus. Brain Res 335:365-367.

Sanders K.M. (1984): Role of prostaglandins in regulating gastric motility. Am J Physiol 247:G117-G126.

Saper C.B., Breder C.D. (1994): The neurologic basis of fever. zzzz 330:1880-1886.

Saperas E., Yang H., Rivier C., Taché Y. (1990): Central action of recombinant interleukin-1 to inhibit acid secretion in rats. Gastroenterology 99:1599-1606.

Saperas E., Kauffman G., Taché Y. (1991): Role of central prostaglandin E2 in the regulation of gastric acid secretion in the rat. Eur J Pharmacol 209:1-7.

Saperas E., Cominelli F., Taché Y. (1992a): Potent inhibition of gastric acid secretion by intravenous interleukin-1 beta and -1 alpha in rats. Peptides 13:221-226.

Saperas E., Yang H., Taché Y. (1992b): Interleukin-1 beta acts at hypothalamic sites to inhibit gastric acid secretion in rats. Am J Physiol 263:G414-G418.

Saperas E., Taché Y. (1993): Central interleukin-1 beta-induced inhibition of acid secretion in rats: Specificity of action. Life Sci 13:221-226.

Saperas E., Taché Y. (1994): Interleukin-1 receptor antagonist does not prevent endotoxin-induced inhibition of gastric acid secretion in rats. Dig Dis Sci 39:152-156.

Saphier D., Ovadia H. (1990): Selective facilitation of putative corticotropin-releasing factor-secretig neurones by interleukin-1. Neurosci Lett 114:283-288.

Sapolsky R., Rivier C., Yamamoto G., Plotsky P., Vale W. (1987): Interleukin-1 stimulates the secretion of hypothalamic corticotropin releasing factor. Science 238:522-524.

Selye H. (1936): A syndrome produced by diverse nocuous agents. Nature 138:32-40.

Sheldon R.J., Jiang Q., Porreca F., Fisher L.A. (1990): Gastrointestinal motor effects of corticotropin-releasing factor in mice. Regul pept 28:137-151.

Shibasaki T., Yamauchi N., Hotta M., Imaki T, Oda T., Ling N., Demura H. (1991): Interleukin-1 inhibits stress-induced gastric erosion in rats. Life Sci 48:2267-2273.

Shibata M., Leffler C.W., Busija D.W. (1990): Recombinant human interleukin 1 alpha dilates pial arterioles and increases cerebrospinal fluid prostanoids in piglets. Am J Physiol 259:H1486-H1491.

Singh V.K., Leu S.J. (1990): Enhancing effect of corticotropin-releasing neurohormone on the production of interleukin-1 and interleukin-2. Neurosci Lett 120:151-154.

Sirko S., Bishai I., Coceani F. (1989): Prostaglandin formation in the hypothalamus in vivo: Effect of pyrogens. Am J Physiol

256:R616-R624.

Skofitsch G., Hamill G.S., Jacobowitz D.M. (1984): Capsaicin depletes corticotropin-releasing factor-like immunoreactive neurons in the rat spinal cord and medulla oblongata. Neuroendocrinol 38:514-517.

Sobel D.O. (1990): Corticotropin releasing hormone and arginine vasopressin stimulation of ACTH and substance P in human mononuclear leukocytes. Endocrine Res 16:283-292.

Staumont G., Fioramonti J., Frexinos J., Bueno L. (1990): Oral prostaglandin E2 analogues induce intestinal migration motor complexes after a meal in dogs. Evidence for a central mechanism. Gastroenterology 98:888-893.

Stein J., Zeuzem S., Uphoff K., Laube H. (1994): Effects of prostaglandins and indomethacin on gastric emptying in the rat. Prostaglandins 47:31-40.

Stephanou A., Jessop D.S., Knight R.A., Lightman S.L. (1990): Corticotropin-releasing factor-like immunoreactivity and mRNA in human leukocytes. Brain Behav Immun 4:67-73.

Sternini C. (1992): Enteric and visceral afferent CGRP neurons. Targets of innervation and differential expression patterns. Ann N Y Acad Sci 657:170-186.

Suda T., Tomori N., Tozawa F., Demura H., Shizume K., Mouri T., Miura Y., Sasano N. (1984a): Immunoreactive corticotropin and

corticotropin-releasing factor in human hypothalamus, adrenal, lung cancer, and pheochromocytoma. J Clin Endocrinol Metab 58:919-924.

Suda T., Tomori N., Tozawa F., Mouri T., Demura H., Shizume K. (1984b): Distribution and characterization of immunoreactive corticotropin-releasing factor in human tissues. J Clin Endocrinol Metab 59:861-866.

Suda T., Tozawa F., Ushiyama T., Sumitomo T., Yamada M., Demura H. (1990): Interleukin-1 stimulates corticotropin-releasing factor gene expression in rat hypothalamus. Endocrinology 126:1223-1228.

Sütő G., Király A., Taché Y. (1994a): Laparotomy-induced inhibition of gastric emptying is mediated by medullary release of oxytocin in rats. Gastroenterology 106:A388 (Abstract).

Sütő G., Király A., Taché Y. (1994b): Interleukin 1 beta inhibits gastric emptying in rats: Mediation through prostaglandin and corticotropin releasing factor. Gastroenterology 106:1568-1575.

Sütő G., Király A., Plourde V., Taché Y. (1996): Intravenous interleukin-1 beta-induced inhibition of gastric emptying: Involvement of central corticotropin-releasing factor and prostaglandin pathways in rats. Digestion 57:135-140.

Szabo S., Glavin G.B. (1990): Hans Selye and the concept of biologic stress. Ulcer pathogenesis as a historical paradigm. Ann N Y Acad Sci 597:14-16.

Taché Y., Maeda-Hagiwara M., Turkelson C.M. (1987): Central nervous system action of corticotropin-releasing factor to inhibit gastric emptying in rats. Am J Physiol 253:G241-G245.

Taché Y., Garrick T., Raybould H. (1990a): Central nervous system action of peptides to influence gastrointestinal motor function. Gastroenterology 98:517-528.

Taché Y., Yang H. (1990b): Brain regulation of gastric acid secretion by peptides. Sites and mechanism of action. Ann N Y Acad Sci 597:128-145.

Taché Y., Raybould H., Wei J.Y. (1991): Central and peripheral actions of calcitonin gene-related peptide on gastric secretory and motor function. Adv Exp Med Biol 298:183-198.

Taché Y. (1992a): Inhibition of gastric acid secretion and ulcers by calcitonin gene-related peptide. Ann N Y Acad Sci 657:240-247.

Taché Y., Saperas E. (1992b): Potent inhibition of gastric acid secretion and ulcer formation by centrally and peripherally administered interleukin-1. Ann N Y Acad Sci 659:353-368.

Taché Y., Monnikes H., Bonaz B., Rivier J. (1993): Role of CRF in stress-related alterations of gastric and coloniuc motor function. Ann N Y Acad Sci 697:233-243.

Takao T., Tracey D.E., Mitchell W.M., De Souza E.B. (1990):

Interleukin-1 receptors in mouse brain: Characterization and neuronal localization. Endocrinology 127:3070-3078.

Takeuchi K., Takehara K., Ohuchi T. (1996): Diethyldithiocarbamate, a superoxid dismutase inhibitor, reduces indomethacin-induced gastric lesions in rats. Digestion 57:201-209.

Tang Y.M., Han C.D., Fiscus R.R., Wang X. (1997): Increase of calcitonin gene-related peptide (CGRP) release and mRNA levels in endotoxic rats. Shock 7:225-229.

Terao A., Oikawa M., Saito M. (1993): Cytokine-induced change in hypothalamic norepinephrine turnover: Involvement of corticotropin releasing hormone and prostaglandins. Brain Res 622:257-261.

Terao A., Kitamura H., Asano A., Kobayashi M., Saito M. (1995): Roles of prostaglandins D2 and E2 in interleukin-1 induced activation of norepinephrine turnover in the brain and peripheral organs of rats. J Neurochem 65:2742-2747.

Theodoru V., Eutamene H., Fioramonti J., Junien J.L., Buéno L. (1994): Interleukin 1 induces a neurally mediated colonic secretion in rats: Involvement of mast cells and prostaglandins. Gastroenterology 106:1493-1500.

Thiefin G., Leung F.W., Tache Y., Guth P.H. (1989): Dissociated effects of corticotropin-releasing factor on acid secretion and blood flow. Am J Physiol 256:G412-G417.

Tilders F.J.H., DeRijk R.H., Van Dam A-M., Vincent V.A.M., Schotanus K., Persoons J.H.A. (1994): Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: Routes and intermediate signals. Psychoneuroendocrinology 19(2):209-232.

Tsagarakis S., Gillies G., Rees L.H., Besser M., Grossmann A.B. (1989): Interleukin-1 directly stimulates the release of corticotropin releasing factor from rat hypothalamus. Neuroendocrinol 49:98-101.

Uehara A., Gottschall P.E., Dahl R.R., Arimura A. (1987): Interleukin-1 stimulates ACTH release by an indirect action which requires endogenous corticotropin releasing factor. Endocrinology 121:1580-1582.

Uehara A., Okumura T., Sekiya C., Okamura K., Takasugi Y., Namiki M. (1989): Interleukin-1 inhibits the secretion of gastric acid in rats: Possible involvement of prostaglandin. Biochem Biophys Res Comm 162:1578-1584.

Uehara A., Okumura T., Kitamori S., Takasugi Y., Namiki M. (1990): Interleukin-1: A cytokine that has potent antisecretory and anti-ulcer actions via the central nervous system. Biochem Biophys Res Comm 173:585-590.

Uehara A., Okumura T., Kitamori S., Shibata Y., Harada K., Okamura K., Takasugi Y., Namiki M. (1992a): Gastric antisecretory and antiulcer actions of interleukin-1. J Clin Gastroenterol

14:S149-S155.

Uehara A., Okumura T., Tsuji K., Taniguchi Y., Kitamori S., Takasugi Y., Namiki M. (1992b): Evidence that gastric antisecretory action of lipopolysaccharide is not due to a toxic effect on gastric parietal cells. Dig Dis Sci 37:1039-1044.

Ulich T.R., Guo K., Irwin B., Remick D.G., Davatelis G.N. (1990): Endotoxin-induced cytokine gene expression in vivo: II. Regulation of tumor necrosis factor and interleukin-1 alpha/beta expression and suppression. Am J Pathol 137:1173-1185.

Vale W., Spiess C., Rivier C., Rivier J. (1981): Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394-1397.

van Gool J., van Vugt H., Helle M., Aarden L. (1990): The relation among stress, adrenalin, interleukin 6 and acuta phase proteins in the rat. Clin Immunol Immunpathol 57:200-210.

van Miert A.S.J.P.A.M., Kaya F., van Duin C.T.M. (1992): Changes in food intake and forestomach motility of dwarf goats by recombinant bovine cytokines (IL-1 beta, IL-2) and IFN-gamma. Physiol Behav 52:859-864.

Vine W., Beaumont K., Gedulin B., Pittner R., Moore C.X., Rink T.J., Young A.A. (1996): Comparison of the in vitro and in vivo pharmacology of adrenomedullin, calcitonin gene-related peptide and amylin in rats. Eur J Pharmacol 314:116-121.

Wallace J.L. (1987): Gastrointestinal plasma leakage in endotoxic shock. Inhibition by prostaglandin E2 and by platelet-activating factor antagonist. Can J Physiol Pharmacol 65:1428-1432.

Wallace J.L., Keenan C.M., Mugridge K.G., Parente L. (1990): Reduction of the severity of experimental gastric and duodenal ulceration by interleukin-1 beta. Eur J Pharmacol 186:279-284.

Wallace J.L., Keenan C.M., Cucala M., Mugridge K.G., Parente L. (1992): Mechanisms underlying the protective effects of interleukin-1 in experimental nonsteroidal anti-inflammatory drug gastropathy. Gastroenterology 102:1176-1185.

Walter J.S., Meyers P., Krueger J.M. (1989): Microinjection of interleukin-1 into brain: Separation of sleep and fever responses. Physiol Behav 45:169-176.

Wan W., Janz L., Vriend C.Y., Sorensen C.M., Greenberg A.H., Nance D.M. (1993): Differential induction of c-fos immunoreactivity in hypothalamus and brain stem nuclei following central and peripheral administration of endotoxin. Brain Res Bul 32:581-587.

Wang D., Nagpal M.L., Calkins J.H., Chang W., Sigel M.M., Lin T. (1991): Interleukin-1 beta induces interleukin-1 alpha messenger ribonucleic acid expression in primary cultures of Leydig cells. Endocrinology 129:2862-2866.

Wang X., Han C.D., Fiscus R.R., Qi M., Jones S.B. (1991):

Hypotension- and endotoxin-induced alterations in calcitonin gene-related peptide: Modulation by dexamethasone. Circ Shock 34:217-223.

Wang X., Han C., Yang L., Chen M., Fiscus R.R. (1992a): Ibuprofen, indomethacin, and high-dose aspirin, but not low-dose aspirin or imidazole, inhibit CGRP elevations in plasma during endotoxicosis. Ann N Y Acad Sci 657:502-504.

Wang X., Jones S.B., Zhou Z., Han C., Fiscus R.R. (1992b): Calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) levels are elevated in plasma and decreased in vena cava during endotoxin shock in the rat. Circ Shock 36:21-30.

Wang X., Han C., Jones S.B., Yang L., Fiscus R.R. (1995): Calcitonin gene-related peptide release in endotoxicosis may be mediated by prostaglandins. Shock 3:34-39.

Warner S.J.C., Auger K.R., Libby P. (1987): Interleukin-1 induces inteleukin-1. II. Interleukin-1 induces production of interleukin-1 by adult human vascular endothelial cells in vitro. J Immunol 139:1911-1917.

Watanabe T.J., Morimoto A., Sakata Y., Murakami N. (1990): ACTH response induced by interleukin-1 is mediated by CRF secretion stimulated by hypothalamic PGE. Experientia 46:481-484.

Watanabe H., Sasaki S., Takebe K. (1991): Evidence that intravenous administration of interleukin-1 stimulates corticotropin releasing hormone secretion in the median eminence of freely moving rats: estimation by push-pull perfusion.

Neurosci Lett 133:7-10.

Watkins L.R., Wiertelak E.P., Goehler L.E., Mooney-Heiberger K., Martinez J., Furness L., Smith K.P., Maier S.F. (1994): Neurocircuitry of illness-induced hyperalgesia. Brain Res 639:183-199.

Watkins L.R., Goehler L.E., Relton J.K., Tartaglia N., Silbert L., Martin D., Maier S.F. (1995): Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci Lett 183:27-31.

Westphal O., Jann K., Himmelspach K. (1983): Chemistry and immunochemistry of bacterial lipopolysaccharides as cell wall antigens and endotoxins. Prog Allergy 33:9-16.

Whittle B.J.R., Boughton-Smith N.K., Hutcheson I.R., Esplugues J.V., Wallace J.L. (1987): Increased intestinal formation of Paf in endotoxin-induced damage in the rat. Br J Pharmac 92:3-4.

Wirthlin D.J., Cullen J.J., Spates S.T., Conklin J.L., Murray J., Caropreso D.K., Ephgrave K.S. (1996): Gastrointestinal transit during endotoxaemia: The role of nitric oxide. J Surg Res 60:307-311.

Wong M.-L., Licinio J. (1994): Localization of interleukin 1 type I receptor mRNA in rat brain. Neuroimmunomodulation 1:110-115.

Woudenberg A.A., Wiegant V.M. (1992): Endorphin-like

immunoreactivities in uncultured and cultured human peripheral blood mononuclear cells. Life Sci 50:705-714.

Yan H.Q., Banos M.A., Herregodts P., Hooghe R., Hooghe-Peters E.L. (1992): Expression of interleukin (IL)-1 beta, IL-6 and their respective receptors in normal rat brain and after injury. Eur J Immunol 22:2963-2971.

Zittel T.T., Rothenhofer I., Meyer J.H., Raybould H.E. (1994): Small intestinal capsaicin-sensitive afferents mediate feedback inhibition of gastric emptying in rats. Am J Physiol 267:G1142-G1145.

Zou Z.Z., Jones S.B. (1993): Involvement of central vs. peripheral mechanisms in mediating sympathoadrenal activation in endotoxic rats. Am J Physiol 265:R683-R688.

Zuckerman S.H., Shellhaas J., Butler L.D. (1989): Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: Effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. Eur J Immunol 19:301-305.

9. Papers, abstracts and book chapters.

9.1. Papers.

1989

1. **Sütő G.**, Garamszegi M., Jávor T., Vincze Á., Mózsik Gy.: Similarities and differences in the cytoprotection induced by PGI_2 and β-carotene in experimental ulcers. Acta Physiol. Hung. 73:155-158 (1989)
2. Mózsik Gy., Garamszegi M., **Sütő G.**, Vincze Á., Jávor T.: A pharmacological approach to cellular mechanisms of PGI_2-induced gastric cytoprotection on ethanol-induced gastric mucosal damage in rats. Acta Physiol. Hung. 73:207-211 (1989)
3. Garamszegi M., Jávor T., **Sütő G.**, Vincze Á., Tóth Gy., Mózsik Gy.: Effect of atropine, $PGF_{2\alpha}$, and cimetidine on the β-carotene-induced gastric cytoprotection in ethanol-treated rats. Acta Physiol. Hung. 73:221-224 (1989)
4. Vincze Á., Garamszegi M., Jávor T., **Sütő G.**, Tigyi A., Tóth Gy., Zsoldos T., Mózsik Gy.: The free radical mechanisms in β-carotene-induced gastric cytoprotection in HCl model. Acta Physiol. Hung. 73:351-355 (1989)
5. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Tóth Gy., Vincze Á.: Cellular energy status of the gastric mucosa and gastric mucosal prevention by vitamin A in indomethacin treated rats. Int. J. Tiss. React. 11: 65-71 (1989)

1990

6. Garamszegi M., Németh A., Patty I., Tárnok F., Mózsik Gy., Vincze Á., **Sütő G.**, Jávor T.: Comparison of the ulcer healing effect of different cytoprotective drugs in gastric ulcer patients. Acta Physiol. Hung. 75(Suppl.):127-128 (1990)

7. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., Németh A., **Sütő G.**, Vincze Á.: Chemicals (alcohol, NaOH, NaCl, HCl)-induced changes in the gastric mucosal membrane-bound ATP-dependent energy systems. Acta Physiol. Hung. 75(Suppl.):217-218 (1990)

8. Mózsik Gy., Király Á., Garamszegi M., Jávor T., Nagy L., Németh A., **Sütő G.**, Vincze Á.: Gastric cytoprotection mediating in SH groups is failed by surgical vagotomy. Acta Physiol. Hung. 75(Suppl.):219-220 (1990)

9. **Sütő G.**, Garamszegi M., Jávor T., Nagy L., Németh A., Vincze Á., Mózsik Gy.: Biochemical background of cimetidine-induced gastric mucosal prevention on rats treated with HCl (actions of cytoprotective and antisecretory doses). Acta Physiol. Hung. 75(Suppl.):259-260 (1990)

10. Vincze Á., Garamszegi M., Jávor T., Nagy L., Németh A., **Sütő G.**, Mózsik Gy.: Biochemical background of β-carotene-induced gastric cytoprotection in rat treated with HCl. Acta Physiol. Hung. 75(Suppl.):293-294 (1990)

11. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., Patty I., **Sütő G.**, Vincze Á.: A biochemical and pharmacological approach to the genesis of ulcer disease II. A model study of stress induced injury to gastric mucosa in rats. Annals N.Y. Acad. Sci. 517:264-281 (1990)

12. Nagy L., Mózsik Gy., Vincze Á., **Sütő G.**, Hunyadi B., Rinfel J., Past T., Jávor T.: Effects of a novel Hungarian antacid containing Al and Mg (Tisacid^{R}) on mucosal prostaglandin generation and oxygen free radicals in normal rats. Drugs Exp. Clin. Res. 16:197-203 (1990)

1991

13. Vincze Á., Garamszegi M., Jávor T., **Sütő G.**, Tóth Gy., Zsoldos T., Mózsik Gy.: The role of oxygen related free radicals in β-carotene and prostacyclin induced gastric cytoprotection in rats. Eur. J. Gastroenterol. and Hepatol. 3:175-179. (1991)

14. Mózsik Gy., **Sütő G.**, Garamszegi M., Jávor T., Nagy L., Vincze Á., Zsoldos, T.: Oxygen free radicals and gastric mucosal damage in rats treated with ethanol and HCl. Eur. J. Gastroenterol. and Hepatol. 3:757-761 (1991)

15. Mózsik Gy., Király Á., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Tóth Gy., Vincze Á.: Failure of prostacyclin, β-carotene, atropine, and cimetidine to produce gastric cyto- and general mucosal protection in surgically vagotomized rats. Life Sciences 49(19):1383-1389 (1991)

1992

16. Mózsik Gy., **Sütő G.**, Vincze Á.: Correlations between the acute chemical and surgical vagotomy-induced gastric mucosal biochemistry in rats. J. Clin. Gastroenterol. 14(Suppl.1):S135-S139 (1992)

17. Mózsik Gy., Király Á., Garamszegi M., Jávor T., Nagy L., Németh A., **Sütő G.**, Vincze Á.: Mechanisms of vagal nerve in the gastric mucosal defence: unchanged gastric emptying and increased vascular permeability. J. Clin. Gastroenterol. 14(Suppl.1):S140-S144 (1992)

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

19. Vincze Á., Király Á., **Sütő G.**, Mózsik Gy.: Acute surgical vagotomy (ASV) is an aggressor of gastric mucosa? Acta Physiol. Hung. Acta Physiol. Hung. 80:197-205 (1992)

20. Király Á., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: Effect of acute (ASV) and chronic (CSV) surgical vagotomy on the vascular permeability of the gastric mucosa after 96% ethanol (ETOH) treatment in rats. Acta Physiol. Hung. 80:221-226 (1992)

21. Király Á., Matus Z., **Sütő G.**, Vincze Á., Mózsik Gy.: Correlation between the β-carotene induced gastric cytoprotective effect and its gastric mucosal level in indomethacin-treated rats. Acta Physol. Hung. 80:215-220 (1992)

22. **Sütő G.**, Király Á., Vincze Á., Jávor T., Mózsik Gy.: Effect of acute surgical vagotomy (ASV) on the mucosal content of 6-keto-$PGF_{1\alpha}$, PGE_2 and glutathione (GSH) after intragastric 96% ethanol (ETOH) treatment in rats. Acta Physiol. Hung. 80:207-213 (1992)

1993

23. Mózsik Gy., Karádi O., Király Á., Matus Z., **Sütő G.**, Tóth Gy., Vincze, Á.: Vagal nerve and the gastric mucosal defense. J.Physiol.(Paris) 87:329-334 (1993)

24. Vincze Á., Király Á., **Sütő G.**, Mózsik Gy.: Changes of gastric mucosal biochemistry in ethanol-treated rats with and without acute surgical vagotomy. J.Physiol.(Paris) 87:339-341 (1993)

25. Vincze Á., Garamszegi M., Karádi O., Király Á., Nagy L., **Sütő G.**, Tóth Gy., Mózsik Gy.: Cellular energy status of the gastric mucosa and gastric mucosal damage prevention by vitamin A in indomethacin treated rats. Exp. Clin. Gastroenterol. 3:199-205 (1993).

26. Mózsik Gy., Garamszegi M., Karádi O., Király Á., Nagy L., **Sütő G.**, Vincze Á.: Correlation between the gastric mucosal biochemistry, vascular permeability and mucosal protection produced by cytoprotective and antisecretory doses of atropine and cimetidine in rats treated with indomethacin. Exp. Clin. Gastroenterol. 3:205-215 (1993)

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

1994

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

29. Plourde V., Quintero E., **Sütő G.**, Coimbra C., Taché Y.: Delayed gastric emptying induced by inhibitiors of nitric oxide synthase in rats. Eur. J. Pharmacol. 256:125-129 (1994)

30. Taché Y., Yoneda M., Kato K., Király Á., **Sütő G.**, Kaneko H.: Intracisternal thyrotropin-releasing hormone-induced vagally mediated gastric protection against ethanol lesions: Central and peripheral mechanisms. J. Gastroenterol. Hepatol. 9:S29-S35 (1994)

31. Király Á., **Sütő G.**, Livingston EH., Guth PH., St.Pierre S., Taché 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)

32. **Sütő G.**, Király Á., Taché Y.: Interleukin-1β inhibits gastric emptying in rats. Mediation through prostaglandin and corticotropin-relasing factor. Gastroenterology 106:1568-1775 (1994)

1996

33. **Sütő G.**, Király Á., Plourde V., Taché Y.: Intravenous interleukin-1β-induced inhibition of gastric emptying: involvement of central corticotropin-releasing factor and prostaglandin pathways in rats. Digestion 57: 135-140 (1996)

1997

34. Király Á., **Sütő G.**, Guth P.H., Taché Y.: 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)

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

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

1998

36. Király Á., **Sütő G.**, Guth P.H., Taché Y.: 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)

9.2. Abstracts.

1985

1. Mózsik Gy., Czeglédi B., Jávor T., **Sütő G.**, Tigyi A., Zsoldos T., Vincze Á.: Correlations between the free radicals and membrane-dependent energy systems in ethanol-induced gastric mucosal damage in rats. Dig. Dis. Sci. 30:390 (1985)

1986

2. **Sütő G.**, Czeglédi B., Vincze Á., Zsoldos T., Ezer E., Mózsik Gy.: Correlations between the gastric cytoprotective effect of a new antirheumatic agent (RGH-2961) and the free radical mechanisms in the ethanol-induced gastric mucosal damage in rats. 2nd World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, Abstract Book, p.A371 (1986)
3. Vincze Á., Czeglédi B., **Sütő G.**, Zsoldos T., Mózsik Gy.: Mucosal protective effects of atropine, $PGF_{2\alpha}$, cimetidine, and gastric mucosal superoxide dismutase (SOD) activity and gastric ulcer healing in rats. 2nd World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, Abstract Book, p.A386 (1986)

1988

4. Mózsik Gy., Figler M., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á.: Biochemical background of the development of gastric mucosal damage produced by intragastric administration of ethanol or HCl in the rats. Dig. Dis. Sci. 33:906 (1988)
5. Mózsik Gy., Figler M., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á.: PGI_2 prevents the development of gastric mucosal damage, β-carotene stimulates the repair mechanisms in ethanol-induced gastric mucosal damage in the rats. Dig. Dis. Sci. 33:906 (1988)

1989

6. Garamszegi M., Vincze Á., **Sütő G.**, Tóth Gy., Jávor T., Mózsik Gy.: Modifications of the gastric cytoprotective effect of β-carotene and PGI_2 by atropine and cimetidine in ethanol-treated rats. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.232 (1989)
7. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Tóth Gy., Vincze Á.: Cellular energy status of gastric mucosa and gastric mucosal prevention by vitamin A in rats treated with indomethacin. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.233 (1989)
8. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á.: Biochemical background of the development of indomethacin-induced gastric mucosal lesions. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.234 (1989)
9. Nagy L., Mózsik Gy., **Sütő G.**, Vincze Á., Hunyady B., Rinfel J., Jávor T.: Effects of a novel Al-containing Hungarian antacid (Al-Mg-hydroxy-carbonate) Tisacid^R on the mucosal prostaglandin generation and oxygen free radicals in rats. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.235 (1989)
10. **Sütő G.**, Király Á., Garamszegi M., Jávor T., Nagy L., Vincze

Á., Tóth Gy., Mózsik Gy.: Neural and hormonal influences on β-carotene-induced gastric mucosal cytoprotection in rats. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.236 (1989)

11. Vincze Á., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Tóth Gy., Mózsik Gy.: Correlations between gastric mucosal lesions, vascular permeability, and β-carotene-induced gastric cytoprotection in ethanol and indomethacin gastric ulcer models. In Proceedings of 3rd Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Monte-Carlo, p.237 (1989)
12. Mózsik Gy., Király Á., Garamszegi M., Nagy L., **Sütő G.**, Tóth Gy., Vincze, Á. Jávor T.: Vagus in gastric mucosal injury and prevention. Dig. Dis. Sci. 34:1319 (1989)

1990

13. Király Á., Balaskó M., Bódis B., Csontos Zs., Karádi O., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: Acute surgical vagotomy (ASV) causes an increased vascular permeability to chemicals in the rat stomach. Dig. Dis. Sci. 35:1019 (1990)
14. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á.: Mechanisms of intact vagal nerve in the development of gastric cytoprotection by PGI_2, β-carotene and small doses of anticholinergic and H_2-blocking compounds. Dig. Dis. Sci. 35:1020 (1990)
15. Garamszegi M., Németh A., Hunyady B., **Sütő G.**, Vincze Á.,

Mózsik Gy., Jávor T.: Effect of vitamin A and ranitidine in duodenal ulcer patients. Dig. Dis. Sci. 35:1029 (1990)

16. Vincze Á., Garamszegi M., Jávor T., **Sütő G.**, Mózsik Gy., Jávor T.: The prevention of increased vascular permeability is not involved in gastric cytoprotective effect of β-carotene. Dig. Dis. Sci. 35:1033 (1990)
17. Király Á., Garamszegi M., Jávor T., **Sütő G.**, Vincze Á., Tóth Gy., Mózsik Gy.: Synergic effect of β-carotene and selenium on gastric mucosal damage produced by ethanol in rats. Dig. Dis. Sci. 35:1036 (1990)
18. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Tóth Gy., Vincze Á., Zsoldos T.: Mechanisms of gastric mucosal protection: non-sulfhydryl antioxidants are mediators, vagus and adrenal cortex are modulators, scavengers are partly targets and CAMP is an intracellular signal molecule. Dig. Dis. Sci. 35:1036 (1990)
19. Balaskó M., Bódis B., Csontos Zs., Karádi O., Király Á., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: Vagus and gastrointestinal defence in rats treated with indomethacin. Dig. Dis. Sci. 35:1041 (1990)
20. **Sütő G.**, Király Á., Tóth Gy., Vincze Á., Jávor T., Mózsik Gy.: Comparative study on the cytoprotection induced by PGI_2, β-carotene and l-cysteine in experimental ulcer in rats. Dig. Dis. Sci. 35:1043 (1990)
21. Mózsik Gy., Garamszegi M., Nagy L., Németh A., **Sütő G.**, Vincze Á., Jávor T.: Correlations between the development of gastric mucosal lesions, mucosal levels of CAMP and PGE_2 in indomethacin-treated rats. Dig. Dis. Sci. 35(12):1568 (1990)

22. Mózsik Gy., **Sütő G.**, Garamszegi M., Jávor T., Nagy L., Vincze Á., Zsoldos T.: Oxygen free radicals and gastric mucosal damage in rats treated with ethanol or Hcl. Dig. Dis. Sci. 35(12):1568 (1990)

23. Garamszegi M., Németh A., Patty I., Tárnok F., Vincze Á., **Sütő G.**, Jávor T., Mózsik Gy.: Comparison of the ulcer healing effect of different cytoprotective and antisecretory drugs in gastric ulcer patients. Dig. Dis. Sci. 35(12):1568 (1990)

1991

24. Király Á., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: The effect of acute surgical vagotomy (ASV) on the changes in vascular permeability and the glutathione (GSH) content of the gastric mucosa. Zeitschrift für Gastroenterologie 29(4):190 (1991)

25. Mózsik Gy., Jávor T., **Sütő G.**, Vincze Á.: Cholinergic and adrenergic neural influences and membrane-bound ATP-dependent energy systems in the gastric mucosa of the rat with intact vagal nerve. Zeitschrift für Gastroenterologie 29(4):195 (1991)

26. **Sütő G.**, Király Á., Vincze Á., Jávor T., Mózsik Gy.: A new computer assisted method to determine the area of mucosal lesions in the stomach induced by chemicals in rats. Zeitschrift für Gastroenterologie 29(4):205-206 (1991)

27. Vincze Á., Király Á., **Sütő G.**, Tóth Gy., Jávor T., Mózsik Gy.: Biochemical and microvascular changes during β-

carotene-induced cytoprotection. Zeitschrift für Gastroenterologie 29(4):212 (1991)

28. **Sütő G.**, Király Á., Tóth Gy., Vincze Á., Jávor T., Mózsik Gy.: Role of endogenous and exogenous sulfhydryl in β-carotene-induced cytoprotection. Exp. Clin. Gastroenterol. 1:6-7 (1991)

29. Vincze Á., Garamszegi M., Király Á., **Sütő G.**, Tóth Gy., Jávor T., Mózsik Gy.: The β-carotene induced cytoprotection does not depend on the mucosal prostaglandin synthesis and the microvascular injury. Exp. Clin. Gastroenterol. 1:7 (1991)

30. Király Á., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: Effect of acute surgical vagotomy (ASV) on the changes of gastric mucosal vascular permeability and PGE_2 and 6-keto-$PGF_{1\alpha}$ during the development of 96% ethanol-induced gastric mucosal lesions. Exp. Clin. Gastroenterol. 1:8 (1991)

31. Vincze Á., Garamszegi M., **Sütő G.**, Tóth Gy., Jávor T., Mózsik Gy.: Biochemical background of β-carotene-induced gastric cytoprotection in rats treated with HCl. Exp. Clin. Gastroenterol. 1:8-9 (1991)

32. Mózsik Gy., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á.: Subcellular mechanisms of the development of gastric hypersecretion, ethanol-induced gastric mucosal damage and PGI_2-induced gastric cytoprotection. Exp. Clin. Gastroenterol. 1:9 (1991)

33. Király Á., **Sütő G.**, Vincze Á., Jávor T., Mózsik Gy.: Effect of acute surgical vagotomy (ASV) on 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(Suppl.1):39 (1991)

34. Mózsik Gy., Jávor T., Király Á., **Sütő G.**, Vincze Á.: β-carotene-induced gastric mucosal protection is independent of gastric mucosal levels of prostaglandins in indomethacin-treated rats with intact vagal nerve. Digestion 49(Suppl.1):46 (1991)
35. **Sütő G.**, Bódis B., Csontos Zs., Karádi O., Király Á., Balaskó, M., Vincze Á., Jávor T., Mózsik Gy.: Effect of graded doses of epinephrine on the ethanol-induced gastric mucosal damage of rats. Digestion 49(Suppl.1):53 (1991)
36. Vincze Á., Király Á., **Sütő G.**, Tóth Gy., Jávor T., Mózsik Gy.: Correlations between non-protein sulfhydryl, cyclic AMP, prostaglandins and vascular permeability during β-carotene-induced gastric cytoprotection in ethanol model of rats. Digestion 49(Suppl.1):55 (1991)

1993

37. Vincze Á., Karádi O., Király Á., Matus Z., **Sütő G.**, Tóth Gy., Mózsik Gy.: Carotenoids and gastric cytoprotection experimental and clinical observations. Exp. Clin. Gastroenterol. 3:240 (1993)
38. Karádi O., Bódis B., Király Á., **Sütő G.**, Vincze Á., Mózsik Gy.: Effect of acute surgical vagotomy on the indomethacin induced colon mucosal damage in rats. Exp. Clin. Gastroenterol. 3:240-241 (1993)
39. Mózsik Gy., Abdel-Salam O.M.E., Bódis B., Király Á., Karádi O., Nagy L., **Sütő G.**, Szolcsányi J., Vincze Á.: Correlations between the vagal nerve, vascular

permeability and gastric mucosal cAMP in rats treated with HCl, ethanol, acidified aspirin and indomethacin. Exp. Clin. Gastroenterol. 3:235-236 (1993)

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

41. **Sütő G.**, Király Á., Plourde V., Taché Y.: Potent inhibition of gastric emptying by centrally and peripherally administered interleukin-1β in rats. Gastroenterology, 104:A523 (1993)

1994

42. Király Á., **Sütő G.**, Livingston EH., Guth PH., Taché Y.: Peripheral mechanisms involved in gastric hyperemic response to central vagal stimulation in rats. Gastroenterology, 106:A555 (1994)

43. Király Á., **Sütő G.**, Guth PH., Taché Y.: 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)

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

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

46. **Sütő G.**, Király Á., Taché Y.: Laparotomy-induced inhibition

is mediated by medullary release of oxytocin in rats. Gastroenterology, 106:A388 (1994)

1995

47. **Sütő G.**, Király Á., Taché Y: Endotoxin induced inhibition of gastric emptying involves peripheral IL-1ß and CRF in rats. Gastroenterology 108:A2676 (1995)

9.3. Book chapters.

1988

1. Mózsik Gy., Czeglédi B., **Sütő G.**, Vincze Á., Zsoldos T.: Effects of atropine and cimetidine administered in cytoprotective and antisecretory doses. In Oxygen Free Radicals and the Tissue Injury. Matkovics B., Boda D., Kalász H. (Eds.) Akadémiai Kiadó, Budapest, pp.225-234 (1988)
2. **Sütő G.**, Vincze Á., Zsoldos T., Mózsik Gy.: Correlations between free radicals and the development of ethanol-induced gastric mucosal damage. In Oxygen Free Radicals and the Tissue Injury. Matkovics B., Boda D., Kalász H. (Eds.) Akadémiai Kiadó, Budapest, pp.357-364 (1988)
3. Vincze Á., **Sütő G.**, Zsoldos T., Mózsik Gy.: Correlations between free radicals and the prostatcyclin methyl ester-β-cyclodextrin complex (PCCD) -induced gastric cytoprotective effect in experimental ulcer. In Oxygen Free Radicals and the Tissue Injury. Matkovics B.,

Boda D., Kalász H. (Eds.) Akadémiai Kiadó, Budapest, pp.389-395 (1988)

4. Mózsik Gy., Garamszegi M., Jávor T., **Sütő G.**, Vincze Á., Tóth Gy., Zsoldos T.: Correlations between the oxygen free radicals, membrane-bound ATP-dependent energy systems in relation to development of ethanol- and HCl-induced gastric mucosal damage and of β-carotene - induced gastric cytoprotection. In Free Radicals in Digestive Diseases. Tsuchiya M. et al. (Eds.) Elsevier Science Publishers, Amsterdam, pp.111-116 (1988)

1989

5. Mózsik Gy., Figler M., Garamszegi M., Jávor T., Nagy L., **Sütő G.**, Vincze Á., Zsoldos T.: Mechanisms of gastric mucosal cytoprotection. I. Time-sequence analysis of gastric mucosal membrane-bound ATP-dependent energy systems, oxygen free radicals and macroscopically appearance of gastric cytoprotection by PGI_2 and β-carotene in HCl-model of rats. In Medical, Biochemical, and Chemical Aspects of Free Radicals. Hayaishi E., Niki M., Kondo M., Yoshikawa T. (Eds.) Elsevier Science Publishers, Co., Inc., Amsterdam, pp.1421-1425 (1989)

6. Mózsik Gy., Garamszegi M., Figler M., Nagy L., **Sütő G.**, Vincze Á., Zsoldos T., Jávor T.: Mechanisms of mucosal injury in the stomach. II. Time-sequence analysis of gastric mucosal membrane-bound ATP-dependent energy systems, oxygen free radicals and appearance of gastric mucosal damage. In Medical, Biochemical, and Chemical Aspects of Free Radicals. Hayaishi E., Niki

M., Kondo M., Yoshikawa T. (Eds.) Elsevier Science Publishers, Co., Inc., Amsterdam, pp.1427-1431 (1989)

7. Mózsik Gy., **Sütő G.**, Vincze Á., Zsoldos T.: Correlations between the free radicals and membrane-bound energy systems in ethanol-induced gastric mucosal damage in rats. In Ulcer Disease: New Aspects of Pathogenesis and Pharmacology. Szabó S., Pfeiffer C.J. (Eds.) CRC Press, Inc., Boca Raton, U.S.A., Chapter 2, pp.15-27 (1989)

1993

8. Mózsik Gy., Karádi O., Király Á., Matus Z., **Sütő G.**, Vincze Á., Tóth Gy.: 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).

1994

9. Mózsik Gy., Bódis B, Garamszegi M., Karádi O, Király Á., Nagy L., **Sütő G.**, Tóth Gy., Vincze Á.: Role of vagal nerve in the development of gastric mucosal injury and its prevention by atropine, cimetidine, β-carotene and prostacyclin in rats. In Neuroendocrinology of Gastrointestinal Ulceration S. Szabó and Y. Tache (Eds.) G. Glavin (coEd.). (1994).

10. Mózsik Gy., Király Á., Sütő G., Vincze Á.: Increased or

decreased cellular energy liberation as types of natural defense mechanisms of the gastrointestinal tract. In Recent advances in mucosal immunology Eds. Jerry McGhee, Jiri Mestecky, Helena Tlaskalova, Jaroslav Sterzl. Plenum Press, New York (in press).