Brain-gut interactions in inflammatory bowel disease: Mechanisms of anorexia in animal models of experimental colitis

HARVEY P WEINGARTEN PhD

HP WEINGARTEN. Brain-gut interactions in inflammatory bowel disease: Mechanisms of anorexia in animal models of **experimental colitis. Can J Gastroenterol 1996;10(5):329-334.** Physiological processes within the bowel are influenced constantly by signals from other organs, primarily the brain. The mechanisms by which inflammation of the gastrointestinal tract results in anorexia are unknown. Understanding how the inflammation-related signals in the periphery are communicated to the central nervous system and activate cytokine production in the brain remains an enormous challenge. Elucidation of these gut-brain communication mechanisms is essential to the development of appropriate and efficacious treatments for the eating and weight disturbances associated with inflammatory bowel disease.

Key Words: *Anorexia, Brain-gut communication, Cytokines, Inflammatory bowel disease*

Interactions entre le cerveau et l'intestin dans la maladie inflammatoire de l'intestin : mécanismes de l'anorexie dans des modèles animaux de colite expérimentale

RÉSUMÉ : Les processus physiologiques intestinaux subissent une influence constante de signaux provenant d'autres organes et surtout du cerveau. Les mécanismes par lesquels l'inflammation des voies digestives provoque l'anorexie sont encore inconnus. Comprendre les signaux périphériques liés à l'inflammation et comment ils sont communiqués au système nerveux central, conduisant à l'activation de la production des cytokines au cerveau, est un défi énorme. Il est essentiel d'élucider les mécanismes de communication entre le cerveau et l'intestin pour développer des traitements appropriés et efficaces contre les troubles liés à l'alimentation et au poids, associés à la maladie inflammatoire de l'intestin.

The present research originates from the assertion that a complete understanding of the symptomatology and etiology of inflammatory bowel disease (IBD) cannot be derived from investigations of one organ, the bowel, in isolation. Rather, it is acknowledged that physiological processes within the bowel are influenced constantly by signals from other organs, primarily the brain, via neural and hormonal communication routes (Figure 1). On the efferent side, dis-turbances in brain-gut communication may precipitate or maintain inflammation in the bowel, especially in situations related to stress (1). On the afferent side, altered communication between gut and brain must underlie the behavioural symptomatology associated with IBD. This paper focuses on one of these symptoms: the reduction of eating (anorexia) and weight loss associated with IBD, particularly Crohn's disease and ulcerative colitis.
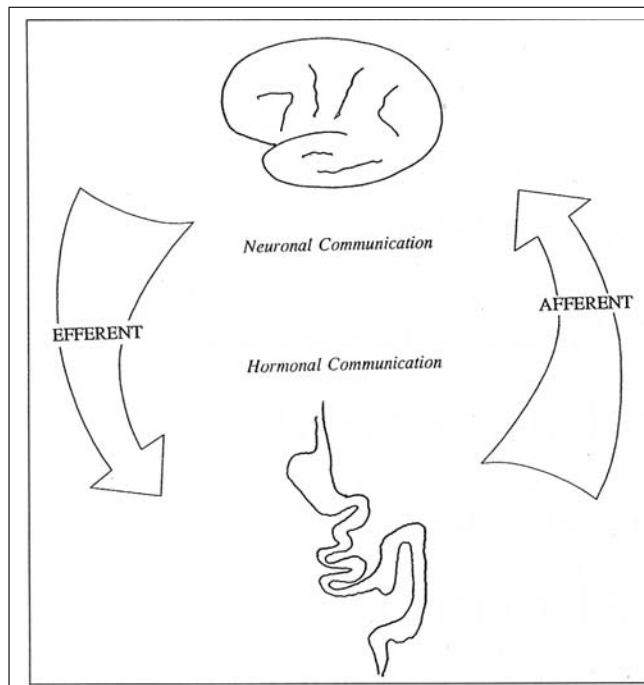
Figure 1) *Schematic representation of bidirectional communication between gut and brain*
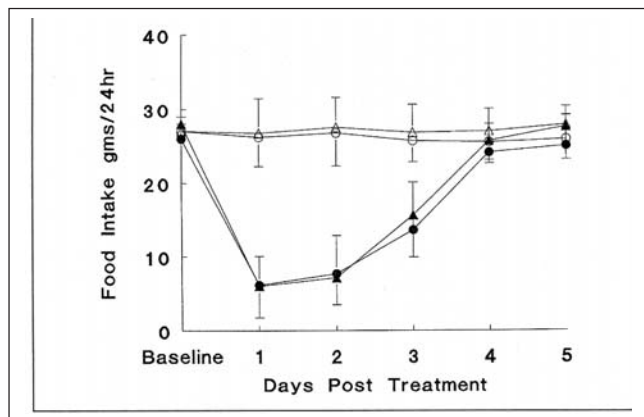

Figure 2) *Profile of anorexia following colon inflammation in the rat. Data shown are levels of 24 h food intakes in the baseline period and for five days after treatment with trinitrobenzene sulphonic acid in ethanol (n=50) (●), 50% ethanol vehicle (n=58) (○), 4% acetic acid (n=25) (▲) or saline vehicle (n=27) (△). Data are group means ±1 SEM. Reprinted with permission from reference 13*

It is estimated that anywhere from 22% (2) to 70% (3) of Crohn's patients exhibit weight loss, a reduction of caloric intake being a major cause. Anorexia is manifested especially during acute exacerbations of IBD, and in children, inadequate nutrition is a major contributor to growth deficiencies (4,5). Weight loss in adult Crohn's patients is so severe that this clinical group is often used as a weight loss control for studies of anorexia nervosa (6). Although malnutrition of the IBD patient is associated with increased morbidity, and amelioration of the nutritional deficiencies is associated with a more positive prognosis (3,7,8), the mechanisms by which inflammation of the gastrointestinal tract results in anorexia are unknown. Because the brain is the organ ultimately responsible for the direction of behaviour, treatment of IBD-associated anorexia and weight loss must involve an understanding of how signals from the inflamed gut are communicated to the brain and identification of the processes that are turned on in the brain upon receipt of these signals to suppress food intake.

ANOREXIA FOLLOWING COLON INFLAMMATION

The initial step in our research strategy was to identify preparations in which the relationship between gut inflammation and anorexia could be productively examined. First, we characterized changes in food intake and body weight in several parasite animal models of gastrointestinal inflammation (*Trichinella spiralis* and *Nippostrongylus brasiliensis*). Although we replicated the observations of Castro et al (9) – that reduced food intake in nematode-infected rats was associated with the period of intestinal inflammation – we found the results variable. More important, in these models, the site of infection was the proximal small intestine, where the parasites invade the epithelium and cause substantial structural damage. Because the proximal duodenum is a site of potential importance in the regulation of feeding (10), it is difficult to distinguish a direct effect of the infection on intestinal feeding systems from that resulting from the host's inflammatory response. Thus, we next concentrated on models of colitis because the distal colon is far removed from the site of putative satiety signals and because colitis is more congruous with the distribution of human IBD.

We established that two animal models of experimental colitis, trinitrobenzene sulphonic acid (TNB) (11) and acetic acid (12), were associated with virtually identical, robust and highly reproducible suppressions of food intake and body weight (Figure 2). Daily caloric intake was reduced by approximately 80%, 70% and 50% on the first three days, respectively, after colitis induction. Food intake normalized by the fourth post-treatment day. Anorexia was associated with a significant weight loss that outlasted the period of food intake suppression (13).

Especially because of the early onset and the large inhibition of food intake it is tempting to suggest that the unwillingness of the colitic animals to eat results simply from the trauma or malaise resulting from the procedures used to induce the inflammation. However, several experiments demonstrate convincingly that neither trauma nor malaise resulting from colitis-inducing treatments is an adequate explanation for the anorexia of these animals (13-15). Consider these findings. First, although the anorexia is fully manifested when rats are maintained on liquid diets, TNB-treated animals demonstrate no reduction of water intake, indicating that they have the capacity for the behaviours necessary to ingest food and that the anorexia is specific to the nutrient. Second, TNB-treated rats manifest the complete profile of anorexia when maintained on a low residue elemental diet, even though the potential malaise associated

with the passage of fecal material over the inflamed segment is not a factor under these feeding conditions. Third, computerized meal pattern analysis reveals that the anorexia of treated animals results specifically from a reduced meal size; TNB-treated animals do not decrease the number of meals initiated during the anorexia. This finding demonstrates that anorexia does not result from a failure to initiate eating but from the elaboration of an exaggerated satiety signal once food has entered the gut. Fourth, TNB-treated rats show no anorexia in sham feeding preparations where the ingested food does not accumulate in the gut. This result indicates that TNB-treated rats have normal appetite and motivation to eat and that the exaggerated satiety signal that terminates meals prematurely in these animals requires gastric distension or stimulation of postgastric tissues. Finally, TNB-treated rats show no elevation of plasma oxytocin, a biological marker of treatments that suppress eating via gastrointestinal malaise.

Results from the TNB and acetic acid colitis models demonstrate a specific, reliable and robust suppression of eating associated with colon inflammation independent of the stimulus used to initiate the inflammatory response. These studies characterize the phenomena of colitis-associated anorexia and validate the use of these animal models for further investigation of the relationship between gut inflammation and eating. In subsequent experiments we sought to answer two fundamental questions regarding the mechanisms of anorexia following colon inflammation: what is the nature of the biological signal that causes the suppression of eating following colon inflammation, and how do the anorexigenic signals from the inflamed colon communicate with the brain?

NATURE OF THE ANOREXIGENIC SIGNAL

The temporal relationship between anorexia and inflammation in the TNB model indicated that food intake suppression was associated with the acute phase of inflammation. In addition, we confirmed early on that the degree of anorexia correlated significantly with the degree of tissue inflammation as shown by myeloperoxidase (MPO) (Figure 3). Thus, we concentrated our search for the anorexigenic signals on biological responses associated with the early parts of the inflammatory cascade. We first defined whether the suppression of eating depended on the production of cyclo-oxygenase or lipoxygenase metabolites of arachidonic acid (13). Leukotriene synthesis was inhibited by repeated intrarectal infusion (10 mg/kg) of the 5′-lipoxygenase inhibitor MK 886. Prostaglandin synthesis was inhibited by repeated intraperitoneal injection (5 mg/kg) of the cyclo-oxygenase inhibitor indomethacin. The results are summarized in Table 1.

The doses of MK 886 and indomethacin selected had no effect on the degree of colon inflammation induced by TNB treatment during the period under examination (five days post-TNB treatment), as shown by the fact that neither treatment changed the degree of MPO activity in colon compared with TNB-treated rats receiving control treat-
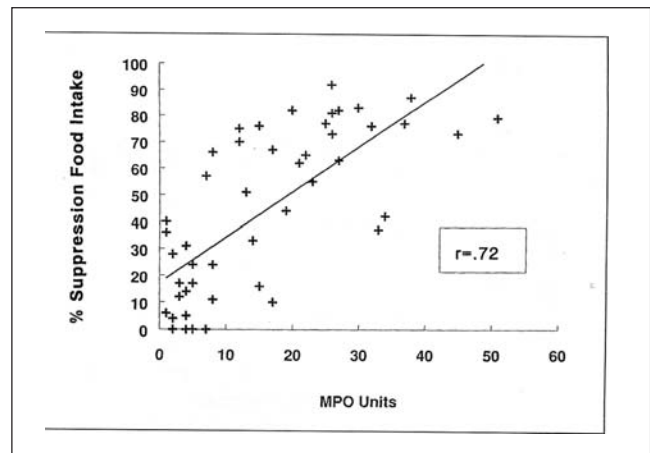
Figure 3) *Correlation between myeloperoxidase (MPO) levels in colon on day 5 and magnitude of anorexia. The degree of anorexia was calculated as the average percentage suppression of 24 h food intake on the first two days after trinitrobenzene sulphonic acid treatment compared with baseline intake*

TABLE 1
Results of a test determining whether the suppression of eating depended on the production of cyclo-oxygenase or lipoxygenase metabolites of arachidonic acid

Treatment	Effect on MPO activity	Effect on tissue levels	Effect on anorexia
MK 886	0	$\downarrow LTB_4$	0
Indomethacin	0	$\downarrow PGE_2$	50% reversal

LTB_4 Leukotriene B_4; MPO Myeloperoxidase; PGE_2 Prostaglandin E_2
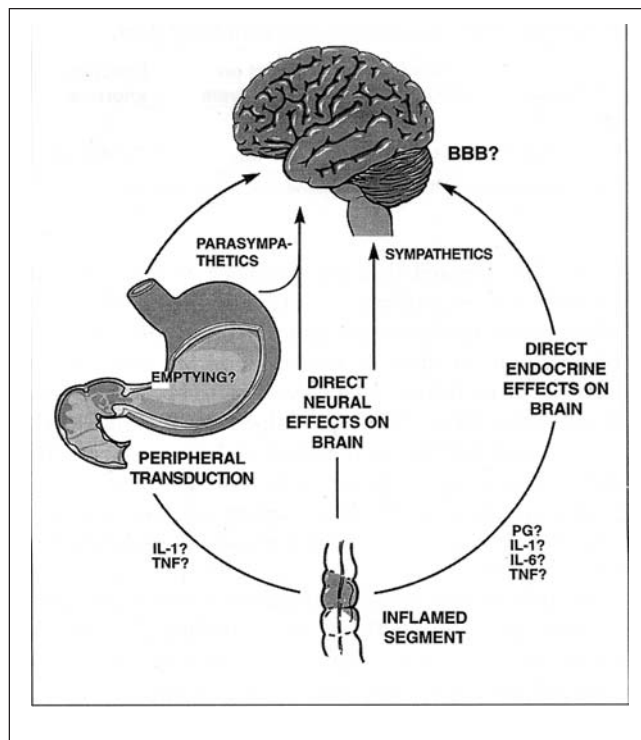
ments. This ensured that any influence of MK 886 and indomethacin on anorexia could be attributed to effects on inflammatory mediators and not on the degree of tissue inflammation. In addition, the ability of MK 886 and indomethacin to reduce colon levels of leukotriene B and prostaglandin E4 in TNB-treated animals to normal levels demonstrated the biological efficacy of the treatments. The critical observation, however, is that inhibition of prostaglandin synthesis significantly reversed the anorexia only following TNB treatments; leukotriene synthesis inhibition was without effect.

We then evaluated the involvement of the acute phase cytokine interleukin-1 (IL-1) in TNB-induced anorexia. Several sets of data implicate IL-1 in anorexia following colon inflammation. First, food intake is suppressed in the first 24 h following colon inflammation and colon levels of IL-1 are already markedly elevated on the first day following TNB administration (16). Second, acute injection of IL-1 suppresses eating (17-19) and chronic administration of IL-1 peripherally results in a profile of anorexia nearly identical to that produced by TNB treatment (20,21). Third, inhibition of eating induced by both TNB (13) and IL-1 (18,22) is attenuated by inhibition of cyclo-oxygenase products of arachidonic acid metabolism.

To evaluate the role of IL-1 we used osmotic minipumps to deliver 24 µg/h recombinant human IL-1 receptor antago-
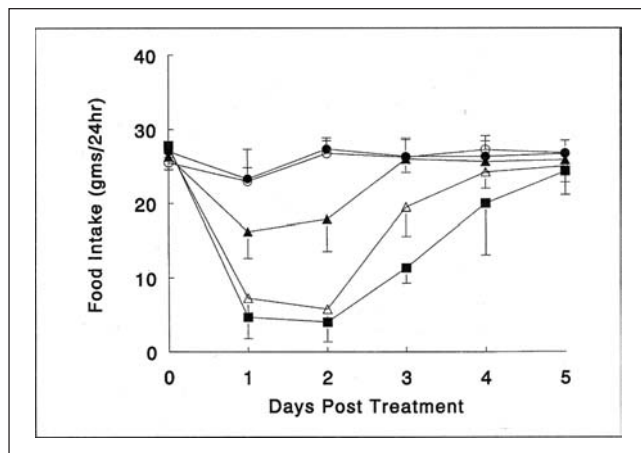
Figure 4) Twenty-four hour food intakes before treatment (day 0) and for five days after treatment with trinitrobenzene sulphonic acid (TNB) + intracerebroventricular (icv) recombinant human interleukin-1 receptor antagonist (rhIL1ra) (▲), TNB + icv saline (△), TNB + subcutaneous rhIL1ra (■), ethanol + icv rhIL1ra (●) or ethanol + icv saline (○). Data are group means ±1 SEM. Reprinted with permission from reference 24

TABLE 2
Results from an examination of total subdiaphragmatic vagotomy (VGx), selective area postrema ablations (APx) and combined area postrema/nucleus of the solitary tract ablations (APx + STA)

Treatment	Effect on MPO	Effect on anorexia
VGx	↑	0
APx	↑	0
APx + STA	↑	0

MPO Myeloperoxidase

effect (29) (Figure 4). These results indicate the necessity of central IL-1 receptors in the expression of the anorexia associated with acute experimental colitis. The suggestion that cytokine expression in the brain, and not in the periphery, is more intimately associated with anorexia following gut inflammation is consistent with a similar conclusion derived from studies evaluating the relative importance of peripheral and central tumour necrosis factor in anorexia and cachexia associated with cancer (25).

COMMUNICATION OF THE ANOREXIGENIC SIGNAL FROM PERIPHERY TO BRAIN

Whatever the exact nature of the anorexigenic signal produced by the inflamed segment, it is obvious that this message must be communicated to the brain in order to affect eating, which forces consideration of the mechanisms by which the anorexigenic signal is transmitted to the central nervous system. The possibilities are outlined in Figure 5.

Inflammation of the colon is associated with production of high levels of acute phase cytokines, such as IL-1 and tumour necrosis factor, both of which suppress eating when injected peripherally (17,26). We have also demonstrated that TNB treatment results in a significant increase in serum IL-6 for at least three days following treatment (27). It is possible that these peripherally released cytokines represent anorexigenic signals that are transmitted to the brain in a classic endocrine manner. This hypothesis requires that peripheral cytokines have access to the brain via either transport mechanisms in the blood-brain barrier (28) or circumventricular organs with porous or absent blood-brain barriers (29). Alteratively, the anorexigenic signal may communicate with the brain indirectly, requiring one or several transductions in the periphery of the signal from the inflamed segment before it is received by brain. This indirect signalling is analogous to the way in which the gut peptide cholecystokinin (CCK) signals satiety (30); CCK released by cells in the proximal duodenum affects neural or smooth muscle elements in the pylorus and, thus, alters vagal afferent traffic to a pattern that signals satiety. This possibility is buttressed by observations that inflammatory mediators (31) and TNB-induced colitis both result in a delayed rate of gastric emptying (14).

As a preliminary examination of the route by which the colitis-produced anorexigenic signals reach the brain, we examined the effects of total subdiaphragmatic vagotomy, selective area postrema ablations and combined area

Figure 5) Schematic representation of the possible mechanisms by which anorexigenic signals from the inflamed segment may be communicated to the brain. BBB Blood-brain barrier; IL Interleukin; PG Prostaglandin; TNF Tumour necrosis factor

nist (23) chronically into either the periphery (subcutaneously) or the brain (intracerebroventricularly). Infusion of this antagonist directly into the brain resulted in a significant reversal of the anorexia and weight loss associated with TNB treatment; peripheral infusion at this dose was without

postrema/nucleus of the solitary tract ablations on the profile of anorexia in the TNB colitis model (27). The results are summarized in Table 2.

Consistent with a previous report by Evangelista and Meli (32) in which peripheral vagal differentiation was produced by capsaicin, both peripheral (subdiaphragmatic) vagotomy and central (area postrema ablations + nucleus solitary tract ablation) vagotomy increased the inflammatory response to intrarectal TNB. However, neither treatment affected the magnitude or time course of the TNB-induced suppression of food intake. These results indicate that expression of anorexia following colon inflammation is not mediated by vagal afferent activity and does not require the entry of an anorexigenic signal into the brain via the area postrema; that although gastric emptying is delayed in association with colon inflammation, this disturbance is not necessary for anorexia; and that although CCK may be involved in the eating suppressive effects of IL-1 (33), it is not a necessary mediator of TNB-induced anorexia.

SUMMARY AND CHALLENGES
FOR FUTURE RESEARCH

The specific biological signals that mediate the anorexic effects of gastrointestinal inflammation remain to be elucidated. Although it is clear that inflammation of the gut leads to the elaboration of multiple inflammatory mediators and cytokines in the periphery, it appears cytokine expression in the central nervous system is critical to the anorexia associated with colitis. There is ample evidence that immune signals that suppress food intake activate cytokine expression in the brain (34). Understanding how the inflammation-related signals in the periphery are communicated to the central nervous system and activate cytokine production in the brain remains an enormous challenge. This problem is nearly identical to an analogous and persisting problem in the understanding of the etiology of fever (35). Elucidation of these gut-brain communication mechanisms is essential to the development of appropriate and efficacious treatments for the eating and weight disturbances associated with IBD.

It is also recognized that IBD is a chronic condition characterized by recurring relapses and acute exacerbations following periods of apparent remission. Studies that focus on the properties and mechanisms of anorexia during the initial inflammatory episode are preliminary but necessary to the study of the mechanisms of chronic reductions of eating and weight that characterize IBD. The successful application of the current research to IBD will require extension of these investigations into models that appropriately mimic the recurring and relapsing nature of human IBD.

REFERENCES

1. McHugh K, Weingarten HP, Khan I, Riddell R, Collins SM. Stress-induced exacerbation of experimental colitis in the rat. Gastroenterology 1993;104:A1051.
2. Farmer RG, Hawk WA, Turnbull RB Jr. Clinical patterns in Crohn's disease: a statistical study of 615 cases. Gastroenterology 1975;68:627-35.
3. Silk DBA, Payne-James J. Inflammatory bowel disease: Nutritional implications and treatment. Proc Nutr Soc 1989;48:355-61.
4. Clark ML. Role of nutrition in inflammatory bowel disease: An overview. Gut 1986;27:72-5.
5. Kirschner BS. Nutritional consequences of inflammatory bowel disease on growth. J Am Coll Nutr 1988;7:301-8.
6. McCallum RW, Grill BB, Lange R, Plansky A, Galss EE, Greenfeld DG. Definition of a gastric emptying abnormality in patients with anorexia nervosa. Dig Dis Sci 1985;30:713-22.
7. Greenberg GR, Flemming CR, Jeejeebhoy IH, Rosenberg IH, Sales D, Tremaine WJ. Controlled trial of bowel rest and nutritional support in the management of Crohn's disease. Gut 1988;29:1309-15.
8. Heatley RV. Assessing the nutritional state in inflammatory bowel disease. Gut 1986;27:61-6.
9. Castro GA, Copeland EM, Dudricks SJ, Johnson LR. Enteral and parenteral feeding to evaluate malabsorption in intestinal parasitism. Am J Trop Med Hyg 1979;28:500-7.
10. Smith GP, Gibbs J. Postprandial satiety. Physiol Psychol 1979;8:179-242.
11. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 1989;96:795-803.
12. MacPherson B, Pfeiffer CJ. Experimental colitis. Digestion 1976;14:424-52.
13. McHugh K, Weingarten HP, Keenan C, Wallace JL, Collins SM. On the suppression of food intake in experimental models of colitis in the rat. Am J Physiol 1993;264:R871-6.
14. McHugh K, Castonguay TW, Collins SM, Weingarten HP. Characterization of suppression of food intake following acute colon inflammation in the rat. Am J Physiol 1993;265:R1001-5.
15. Hansell E, Collins SM, Verbalis JG, Stricker EM, Weingarten HP. Plasma oxytocin levels are not elevated during colitis-induced anorexia in rats. 11th International Conference on the Physiology of Food and Fluid Intake. Oxford, July 28 to 30, 1993.
16. Rachmilewitz D, Simon PL, Schwartz LW, Griswold DE, Fondacaro JD, Wasserman MA. Inflammatory mediators of experimental colitis in rats. Gastroenterology 1989;97:326-37.
17. Dinarello C. Interleukin-1 and interleukin-1 antagonism. Blood 1991;77:1627-52.
18. Hellerstein MK, Meydani SN, Meydani M, Wu K, Dinarello C. Interleukin-1-induced anorexia in the rat. J Clin Invest 1989;84:228-35.
19. Plata-Salaman CR, French-Mullen JMH. Intracerebroventricular administration of a specific IL-1 receptor antagonist blocks food and water intake suppression induced by interleukin-IB. Physiol Behav 1992;51:1277-9.
20. Mrosovsky N, Molony LA, Conn CA, Kluger MJ. Anorexic effects of interleukin-1 in the rat. Am J Physiol 1989;257:R1315-21.
21. Otterness IG, Seymour PA, Golden HW, Reynolds JA, Daumy GO. The effects of continuous administration of murine interleukin-1 in the rat. Physiol Behav 1988;43:797-804.
22. Uehara A, Ishikawa Y, Okumura T, et al. Indomethacin blocks the anorexic action of interleukin-1. Eur J Pharmacol 1989;170:257-60.
23. Eisenberg SP, Evans RJ, Arend WP, et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature 1990;343:341-6.
24. McHugh K, Collins SM, Weingarten HP. Central interleukin-1 receptors contribute to suppression of feeding after acute colitis in the rat. Am J Physiol 1994;266:R1659-63.
25. Tracey KJ, Morgello S, Koplin B, et al. Metabolic effects of cachectin/tumor necrosis factor are modified by site of production. J Clin Invest 1990;86:2014-24.

26. Plata-Salaman CR. Immunomodulators and feeding regulation: A humoral link between the immune and nervous systems. Brain Behav Immun 1989;3:193-213.

27. Weingarten HP, Ladenheim EE, Emond M, Collins SM. Neither subdiaphragmatic vagotomy nor area postrema lesions block anorexia following colon inflammation in the rat. Soc Neurosci 1993;19:240.10. (Abst)

28. Banks WA, Kastin AJ. Blood to brain transport of interleukin links the immune and central nervous systems. Life Sci 1991;48:117-21.

29. Rothwell NJ. Functions and mechanisms of interleukin-1 in the brain. Trends Physiol Sci 1991;12:430-5.

30. Smith GP, Jerome C, Norgren R. Afferent axons in the abdominal vagus mediate satiety of cholecystokinin in rats. Am J Physiol 1985;261:R64-9.

31. Robert A, Olafsson AS, Lancaster C, Zhang WR. Interleukin-1 is cytoprotective, antisecretary, stimulates PGE-2 synthesis by the stomach and retards gastric emptying. Life Sci 1991;48:123-34.

32. Evangelista S, Meli A. Influence of capsaicin-sensitive fibres on experimentally-induced colitis in rats. J Pharm Pharmacol 1989;41:574-5.

33. Daun JM, McCarthy DO. The role of cholecystokinin in interleukin-l-induced anorexia. Physiol Behav 1993;54:237-41.

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

35. Blatteis CM. Neuromodulative actions of cytokines. Yale J Biol Med 1990;63:133-46.

The Scientific World Journal

Gastroenterology Research and Practice

MEDIATORS of INFLAMMATION

Journal of Diabetes Research

Disease Markers

Journal of Immunology Research

International Journal of Endocrinology

PPAR Research

BioMed Research International

Hindawi

Submit your manuscripts at
http://www.hindawi.com

Journal of Ophthalmology

Stem Cells International

eCAM

Evidence-Based Complementary and Alternative Medicine

Journal of Obesity

Journal of Oncology

Computational and Mathematical Methods in Medicine

Behavioural Neurology

Parkinson's Disease

AIDS Research and Treatment

Oxidative Medicine and Cellular Longevity