ANOREXIA AND INFLAMMATION:

FOOD INTAKE STUDIES IN A RAT MODEL OF INTESTINAL INFLAMMATION

BY

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ABSTRACT

This research examines the hypothesis that in rats inflammation of the gastrointestinal tract results in a specific decrease of food intake. A model for examining gastrointestinal inflammation, the trinitrobenzene sulphonic acid model, and its effects on feeding is developed and investigated in the rat. The TNB model results in a predictable and reproducible colonic inflammation that is accompanied by a rapid but transient decrease in food intake. Experiments are presented which: i)investigate if the anorexia is due to nonspecific malaise. ii)investigate, by meal pattern analysis, the profile of food intake displayed by TNB treated animals iii)investigate specifically, which mediators released from the sight of inflammation could be an anorexigenic signal to the central nervous system.

To demonstrate that the anorexia is not due to malaise we have shown that the anorexia: is not specific to the TNB model, is accompanied by no decrease in water intake and develops even when animals are maintained on highly palatable or low residue liquid diets. More importantly animals specifically decrease meal size not meal frequency. Decreased meal size with maintenance of meal frequency is consistent with the generation of abnormal satiety signals and not a "general malaise." These findings show that rats are capable of performing the behaviours necessary for food intake, and do not show specific conditioned taste aversions.

The hypothesis that the generation of abnormal gastric and/or post-gastric satiety

signal is responsible for TNB induced anorexia was also investigated. I demonstrated removal of both gastric and post-gastric satiety signals in a sham feeding preparation resulted in normal intake even in otherwise anorexic animals. I also show that animals with TNB induced colonic inflammation, present with a decreased rate of gastric emptying. These data raise the possibility that abnormal satiety signals, generated as a result of decreased gastric emptying result in decreased meal size and an overall anorexia.

Finally, by specifically inhibiting synthesis of leukotrienes and prostaglandins respectively, or by using an interleukin-1 receptor antagonist (rhIL-1ra), I examined the role of these inflammatory mediators in the anorexia seen in the TNB model of colitis in the rat. I demonstrate that inhibition of prostaglandin synthesis and blockade of interleukin-1 receptors particularly in the brain, result in a significant attenuation of the anorexia. I conclude that the full expression of the anorexia observed in TNB treated animals is dependant on the production of prostaglandins and occupation of centrally located interleukin-1 receptors. Thus, here I specifically describe a model suitable for the investigation of the mechanisms of gastrointestinal inflammation induced anorexia and generally a model that may be used to examine how peripherally generated signals can alter behaviour by communication with the central nervous system.



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# GENERAL INTRODUCTION

Inflammatory Bowel Disease (IBD) is increasing in incidence (7), and is becoming an increasingly large financial burden on the health care system (7). Thus, research in this area is becoming more intensive in an effort to alleviate suffering from the disease and to decrease medical expenditures. IBD consists of two clinically similar, but pathologically distinct, clinical entities: ulcerative colitis (UC) and Crohn's disease (CD). These conditions have both been characterized for some time. Medical journals from as far back as the 19th century have described ulcerative colitis (2, 48, 97). It was in 1932 that Dr. B.B. Crohn coined the term "regional ileitis", which was later renamed Crohn's disease in his honour (26). While their clinical manifestations are similar, the pathological features of the two diseases differ (119).

UC is a non-specific inflammatory disease, predominantly mucosal, extending from the rectum to more proximal areas of the large bowel. UC is not seen in the small bowel, and the gross appearance of UC is that of a continuous involvement of the colon. Ulcers of the mucosa are not always visible macroscopically or on colonoscopic examination. The histology of UC usually shows an inflammatory infiltrate which may extend to the level of the muscularis mucosae, but not below. The infiltrate includes neutrophils (acute stage) as well as macrophages and lymphocytes (chronic stage) (119, 126).

CD can affect any part of the gastrointestinal tract from lips to anus (119, 126).





The histo-pathology of CD includes chronic inflammatory changes that may involve all layers of the bowel wall, and are often associated with granulomas and deep fissuring ulceration (119). The first identifiable pathological lesion of CD is the aphthous ulcer, which can grow to large transverse or round ulcers. Gross examination of CD tissue reveals intersecting longitudinal and transverse ulcers resulting in a "cobblestone" appearance. Transmural inflammation is regularly seen with CD and may include granulomas and lymphoid aggregates with germinal centres. There may also be hyperplasia of neural bundles of the myenteric plexus in CD.

Diagnosis of either CD or UC can be made on the basis of contrast X-rays or direct visualization with fibre optic scope, however, biopsy and histological examination is the most definitive method (126, 127). The underlying causes of both forms of IBD have not been identified. It is widely accepted however, that the gastrointestinal inflammation seen in IBD is either an appropriate response to some as yet unidentified antigen or is the result of inappropriate inflammation triggered by an hyper-reactive immune system (119).

Inflammation of the gastrointestinal tract in man is accompanied by a reduction in body weight that may reflect decreased food intake (anorexia), impaired nutrient absorbtion, and/or increased metabolic demands (38). Significant weight loss is evident in as many as 70%-80% of patients with Crohns (119). The weight loss in Crohn's patients can be so severe that this population has been used as a positive control group in studies of anorexia nervosa (77). As a result of their decreased food intake, IBD patients often fail to eat the recommended daily allowance of many vitamins and



nutrients (57) and, as a consequence, are frequently malnourished (21, 22, 38, 47, 49, 101, 103, 105). The impact of prolonged periods of insufficient nutrient intake in Crohn's disease is especially severe in children and adolescents who often fail to grow adequately (6, 21, 63, 105). Thus, in adults as well as children and adolescents, malnutrition contributes to the mortality and morbidity of IBD (7, 28, 52, 93). Conversely, correction of malnutrition is associated with a reduced probability of surgery and decreased need for medication (31, 37, 43, 68, 119). There are also studies indicating that improving the nutritional status of a patient can be as effective in the treatment of Crohn's disease as steroid therapy (57, 91, 108). Thus, the importance of nutrition in IBD cannot be overstated. Several hypotheses have been forwarded to explain the impact of improved nutrition on the pathophysiology of IBD (53, 57, 108).

The mechanisms underlying the anorexia associated with intestinal inflammation are unknown. It is recognized that both the proximal (24, 69, 121) and lower (65) regions of the gastrointestinal tract are sources of signals controlling food intake (80, 124, 142). The specific mechanisms whereby the gut signals the brain to influence food intake are incompletely understood but may involve the release of hormones, changes in motor function or activation of enteric nerves. It is becoming increasingly evident that inflammation perturbates function in virtually all tissues of the gut wall ranging from the epithelium (106) to the motor apparatus (19, 123, 137). In addition, inflammation is associated with changes in the neuropeptide content of the gut wall (8) and structural changes in the submucous and myenteric nerve plexi of IBD patients

(32, 33, 120, 129). These changes may result in the altered contractility of GI smooth muscle observed at sights remote from the inflammation (46). **It is therefore reasonable to postulate that altered food intake could arise as a result of the impact of inflammation on gastrointestinal signals involved in the control of normal food intake. Alternatively, mediators or agents produced by the inflammatory process might signal the gut or brain directly or indirectly, to suppress eating.**

There are several inflammatory peptides known as cytokines that are produced in abundance in the tissues of patients with IBD (17, 45, 70, 102, 115, 128). Some of these cytokines have been demonstrated to cause perturbations in areas of the gastrointestinal tract as well as other systems involved in the control of feeding (83, 128). Specifically, the cytokine IL-1 appears to correlate well with IBD activity, (17) and has been shown to decrease gastric acid secretion, delay gastric emptying, and stimulate the release of CCK (110, 135). Delayed gastric emptying could result in increased satiety due to increased gastric distention with subsequent stimulation of mechano-receptors. Clearly, using IL-1 as an example, there are data to support the hypothesis that factors released from the inflammatory process can alter the mechanisms of food intake control. In the context of IL-1, there is a body of circumstantial evidence upon which to postulate a role for this cytokine in mediating the reduction of food intake in IBD.

Patients with active inflammatory bowel disease often exhibit anorexia. The possibility of a causal link between inflammation and reduction in feeding behaviour is supported by a limited number of studies in animals with intestinal inflammation



secondary to infection by enteric parasites. Enteric parasitic infections are accompanied by marked weight loss (20, 112). Although these parasites disturb a variety of gastrointestinal functions, including both digestive and absorptive function (112), studies using intravenous or enteric feeding in parasitized animals support the claim of Symons (130) that the primary cause of weight loss in these models is decreased food intake (20). Although models of parasite-induced intestinal inflammation are established and exhibit marked reductions in feeding, the complexity of host-parasite interactions limits their use as effective models to investigate inflammation-induced anorexia. The recent availability of simpler models of inflammation that more closely resemble prevalent human inflammatory conditions prompted these studies.

The purpose of this thesis was to determine whether experimental colitis induced by trinitrobenzene sulfonic acid (TNB) in the rat is a suitable model to investigate changes in feeding behaviour that occur in the context of intestinal inflammation and to use the model to explore the underlying mechanisms of this anorexia. Development of a model for investigating inflammation induced anorexia was necessary as there are no established models suitable to study this phenomenon. This thesis will be presented in four chapters. In Chapter 1, I investigate the utility of the TNB model to examine changes in food intake and body weight in rats and determine the specificity of my findings by comparing results with those obtained in a model of colitis induced by acetic acid. After validating the TNB model I address three further experimental questions. In Chapter 2, I test the hypothesis that malaise is an inadequate explanation for the observed decrease in feeding by using various diets and meal pattern analysis.



Finally Chapters 3 and 4 address the issue of specific mechanisms underlying the anorexia by utilizing a sham feeding preparation, measuring gastric emptying and by investigating the extent to which changes in food intake following colon inflammation are due to liberation of inflammatory mediators. These include the cytokines, which are found in increased concentration in the TNB-induced colitis model (12, 14, 104, 139, 145).

# CHAPTER 1.

## INTRODUCTION

Although, several animal models exist which permit us to study the impact of inflammation on gut function, very few studies to date have addressed the changes in food intake that accompany intestinal inflammation. A recent review of animal models of intestinal inflammation suggests that an "ideal" animal model should provide insights into mechanisms of tissue injury and regeneration, factors involved in the initiation, perpetuation and reactivation of intestinal inflammation, and the utility of new therapeutic agents as well as the mode of action of existing treatments (115). An "ideal" model should also be reproducible, predictable, easy to induce and maintain, and possible to use in available/economically viable animal species. The "ideal" model should also resemble the clinical course of IBD in: pathological features (including loss of appetite), profile of inflammatory mediators, therapeutic response to drugs used in human disease and be spontaneously or inducibly relapsing.

None of the currently available animal models of IBD fits all of the criteria as described above. Each model has both good and bad features with respect to the "ideal" model of IBD. For this reason, it is important that a model be selected on the basis of the outcomes that an investigator wishes to assess. Currently available models of inflammatory bowel disease can be divided into two groups, spontaneous and inducible:

Spontaneous models of colitis

Due to limited access and their unpredictable and spontaneous nature, some of the best examples of "IBD-like" conditions in animals are unsuitable for thorough investigation. A specific sub-group of New World marmosets often develop a mild chronic colitis that is spontaneously relapsing in captive animals (71). The most widely investigated of these animals are cotton topped tamarins (CTT). CTT show several similarities in their colitis to human sufferers of IBD: 1)acute relapsing colonic inflammation with increased incidence of adenocarcinoma of the colon. 2)wasting syndrome characterized by weight loss, diarrhoea, anorexia and death. 3)similar histological changes in the colon with thickened mucosa, elongated crypts, and inflammatory cell infiltration. 4)association between stress and illness. 5)improvement following sulfasalazine or 5-lipoxygenase inhibitors (115). Unfortunately there is very limited access available to this endangered species and sacrifice for medical research is rarely sanctioned. While promising as a valuable tool for the investigations of IBD (particularly ulcerative colitis) the limited availability of, and controlled access to, these animals make them unsuitable for the majority of researchers.

Inducible Colitis Models

There are many models of colitis that can be induced in animals. Most of these models involve direct mucosal injury, immune mediated responses or some combination that leads to intestinal inflammation (62). Again, it is important that the characteristics of the particular model be selected for a defined aspect of the disease an investigator wishes to examine. Some of the more frequently used models will be reviewed briefly.



**Peptidoglycan-Polysaccharide**

Pathogenic and normal bacteria in the small and large bowel produce products that have been shown to produce inflammatory responses (136). These bacterial products include formylated oligopeptides, lipopolysaccharide (LPS), and peptidoglycan-polysaccharide (PG-PS) polymers (115). Using genetically susceptible rats Sartor et al have shown that the serosal injection of a suspension of PG-PS leads to chronic inflammatory changes in the bowel, as well as extra-intestinal manifestations (113, 114). Three to 16 weeks after PG-PS injection, 70-80% of Lewis rats had gross histologic and biochemical evidence of granulomatous colonic inflammation, and also developed anaemia, arthritis and hepatic granulomas. The long lasting chronic inflammation in the bowel make PG-PS injection a promising model for further investigation. Unfortunately the purification of PG-PS is difficult and laparotomy is required for serosal injection. Most importantly, the reproducibility of the model still remains in question as it has only been reported by one research group (115).

**Carrageenin and Dextran Sulphate Sodium**

Chronic feeding of carrageenin (an extract of red seaweed) in drinking water leads to mucosal lesions in the colon of guinea pigs and rabbits in a dose and time dependant manner. Rats, mice and monkeys show blunted response to the substance as indexed by the severity of mucosal lesions (75). Inflammation develops as superficial ulceration beginning in the caecum and right colon, chronic and acute inflammatory cell infiltration in the lamina propria, epithelial atrophy and occasional



crypt abscesses. In mice the repeated administration of dextran sulphate sodium (DSS) in drinking water leads to chronic colonic inflammation with mucosal ulceration, infiltration with mononuclear and polymorphonuclear leukocytes, and epithelial dysplasia in the right colon (89). It has been shown that carrageenin induced colitis requires the presence of specific anaerobic bacteria as treatment with metrodizanole attenuates the inflammation (16). A bacterial association has also been suggested for DSS colitis (115). These immune-mediated models warrant further investigation. However, the limited availability of carrageenin and the fact that rats and mice are resistant to its effects show this models' limitations, as investigations using recombinant antibodies in these species are important research tools. The main problem with DSS is the variable results obtained from different researchers, likely using different dosages indicating that further characterization of the model is required.

**Immune Complex Colitis**

In rabbits, the intravenous injection of preformed immune complexes in antigen excess two hrs after the intra-rectal infusion of 1% formalin induced inflammation in the colon (56). Inflammation was characterized by rectal mucosal ulceration and inflammatory cell infiltration of the lamina propria. Inflammation peaked at 2 days after injury, but mild inflammation of the mucosa was observed at 6 weeks (56). This model is additionally good as it shares cytokine profiles similar to that seen in IBD, and rabbit antibodies are readily available for many cytokines (23). Unfortunately rabbits are costly to purchase and house and the chronic nature of this model is still



in question (115). Additionally the feeding habits of rabbits are not very suitable for study.

**TNB/Acetic acid**

In rats, intra-rectal (IR) administration of trinitrobenzene sulfonic acid in 50% ethanol vehicle (TNB) results in immediate, acute inflammatory changes leading to ulceration and eventually chronic inflammation of the distal colon (84, 104, 139). The chronic phase is characterized by the appearance of granulomas and Langhans-type giant cells and has been demonstrated to last for up to 8 weeks (84). The inflammation has been confirmed by: altered histology, high myeloperoxidase activity, decreased glutathione and the increased production of inflammatory mediators (12, 14, 84, 104, 139). The mechanism by which TNB induces inflammation has not been fully elucidated. There are several reports showing TNB is able to covalently bind to a lysine amino grouping and thus modify cell surface proteins. One hypothesis states that sensitized T lymphocytes that have been preexposed to TNB lyse the cells to which the hapten is bound (131). However, this hypothesis does not account for the inflammation induced by the first exposure to TNB. A more plausible explanation is that macrophages digest the hapten bound cells (without previous exposure to the TNB), and are the predominant effector cell in the tissue damage seen with primary TNB treatment (67). Additionally the damage to tissue may be a result of the liberation of oxygen free radicals acting in a cytotoxic fashion. This was illustrated by the demonstration that rat colon and colonocytes are able to produce superoxide,

hydrogen peroxide, and hydroxyl radical in vitro as metabolites of TNB (44). These oxidants in the mucosa could be the trigger for an inflammatory response and lead to tissue injury (145). The appearance of granulomas and Langhans-type giant cells is one attractive feature of this model, as these changes are frequently found in biopsies of Crohn's disease patients tissue (119). Additionally the TNB treated colon resembles Crohn's disease as there is transmural inflammation, skip lesions (cobblestoneing) mast cell and lymphocyte infiltrate and crypt distortion. In review, there are four attractive features to this model:1) the inflammation induced by a single IR infusion of TNB is convenient, predictable and reproducible. 2) rats are relatively inexpensive. 3)damage is seen for at least 8 weeks allowing for the evaluation of treatment effects. 4) histologically relevant especially to the acute phase of Crohn's disease (84).

IR administration of dilute acetic acid to rats, rabbits and guinea pigs induces an acute colitis (36, 72, 117, 145). Tissue MPO levels are significantly elevated indicating PMN infiltration. Tissue levels of arachidonic acid metabolite levels are similar to those seen in human IBD (36, 118). Crypt alterations, diffuse transmural ulceration and non-specific inflammation have all been observed with acetic acid induced colitis in the rat and resemble ulcerative colitis (72). Colitis is not simply due to the chemical insult provided by the acetic acid as IR administration of dilute hydrochloric acid with the same pH or of equally caustic sodium acetate did not lead to the same degree of colonic injury or inflammation seen with acetic acid (146). It would then seem that the protonated form of the acid is required to induce colitis (145). Specifically, the mechanism by which acetic acid induces inflammation in the



rat colon appears to involve the entry of the lipid soluble form of the acid into the epithelium, where it dissociates to liberate protons within the intracellular space. It is likely that massive intracellular acidification is most responsible for the epithelial injury observed (145).

A recent review of both the TNB and acetic acid models suggests the "inflammation induced by acetic acid or TNB results from an initial caustic injury to the colonic epithelial and interstitium, as assessed by the rapid increases in mucosal permeability, edema, and histology" (145). Yamada et al go on to say "TNB and acetic acid are useful models of colitis to study the events that occur at the time of inflammation such as arachidonate metabolism, granulocyte infiltration and metabolism or repair". It must be kept in mind however the limitations of these models. Both TNB and acetic acid induced colitis are induced by an initial caustic mucosal injury. There is little evidence to support a direct chemically induce mucosal injury as a cause of human IBD. Thus, there is a limited usefulness of these models in understanding the events (possibly immune) which lead to induction, relapse and/or maintenance of the chronic phases of human IBD. The well characterized nature of the inflammation and it's time course in the TNB and acetic acid models make them our choice for the following investigations. The purpose of the first series of experiments was to determine if TNB is a suitable model for investigating the food intake changes associated with colonic inflammation. In the study I also used acetic acid induced colitis to determine whether the observed changes in food intake were specific for the stimulus used to induce inflammation.



## GENERAL MATERIAL AND METHODS

*All experiments follow these methods unless otherwise noted

### Subjects

Male Sprague-Dawley albino rats weighing 300-350 g were obtained from Charles-River Laboratories (St. Constant, Quebec, Canada). Rats were housed individually in hanging cages in a colony room with a 12:12 light-dark cycle. Food and water were available ad libitum. A total of ninety animals were used to determine the time course of inflammation in the two experimental colitis models. On day 0, rats received either TNB, acetic acid, or the corresponding control treatments of ethanol or saline each by IR administration. Groups of animals were sacrificed on each of days 1, 3, and 5 post-induction of colitis.

### Induction of Colitis

**TNB model:** Rats were lightly anaesthetized with ether. Then 30mg of 2,4,6-Trinitrobenzenesulphonic acid (TNB) (Eastman Kodak Co., Rochester, NY.), dissolved in 0.25 ml 50% (v/v) ethanol, was infused IR via a PE 60 catheter (84). The tip of the catheter was inserted so the tip was situated 8 cm proximal to the anus. Control infusions consisted of 0.25 ml of the 50% ethanol vehicle only, infused under similar conditions.

**Acetic acid:** In ether-anaesthetized rats, 1 ml of 4% (v/v) acetic acid was infused IR in a manner similar to that described for TNB (117). The colon was



flushed with 1 ml of .15M buffered saline (pH 5.0) for 20 sec after the acetic acid infusion. Control animals received IR infusion of 1 ml of .15M buffered saline only.

### Measurements of Food and Water Intake

Food consisted of powdered rat chow (Purina # 5001) and was available ad libitum. Twenty-four hour food intakes were measured for seven consecutive days prior to, and for five days after, TNB or acetic acid treatments. Powdered rat chow and food cups were weighed, placed in the animals cages, removed twenty-four hours later and weighed again. The intake of the rat was recorded as the difference between the two weights. Any days where there was visibly spilled chow under the cage, that rats food intake was excluded from the data.

### Assessment of Colonic Inflammation

Tissue myeloperoxidase (MPO) activity, a measure directly related to the number and activity of myeloid cell infiltrate in tissue (66, 122), was assayed to monitor the degree of inflammation. Rats were sacrificed by rapid deceleration and cervical dislocation. Then, full thickness tissue samples of approximately 300mg were taken from the colon 8 cm proximal to the rectum, or just adjacent to this area if tissue was badly necrotic. The sample was weighed, minced with scissors for 30 sec, placed in 0.5% hexadecyltrimethylamonium bromide (50 mg tissue per ml) and then homogenised for 20 s using a Kinematica 1650 tissue homogenizer (15). The supernatant was then added to an o-dianisanide solution which changes colour depending on the amount of

MPO in the tissue (15). The rate of change of colour was assessed with a spectrophotometer. Degree of inflammation is reported as units of MPO activity per gram of wet tissue. A unit of MPO is the quantity able to convert 1 mol of hydrogen peroxide to water in 1 min at 22°C.

Results were analyzed with analyses of variance (ANOVA). Where warranted, multiple comparisons were performed using the Studentized range statistic (q) and evaluated according to the Newman-Keuls procedure. A probability level less than 0.05 was accepted as the significance level.

## RESULTS

### Assessment of Inflammation

MPO activity on days 1,3 and 5 following the induction of colitis is shown in Figure 1. On day 1, administration of ethanol caused a small but insignificant increase in MPO activity compared to saline. However, administration of TNB increased MPO activity significantly above that observed with ethanol alone on day 1 ($q_1 = 4.34$, $p < .05$). On days 3 and 5, MPO activity was four times greater in TNB-treated rats than in ethanol controls ($q_3 = 7.83$, $p < .01$ for day 3 and $q_2 = 8.15$, $p < .01$ for day 5). TNB caused macroscopically-evident damage to the colon with necrosis, edema and haemorrhage noted.

Similarly, IR administration of acetic acid increased MPO activity in comparison with saline injection. The difference between acetic acid treated rats and their saline



controls just failed to reach statistical significance on day 1, ($q_1 = 3.43$, $p < .10$) even though MPO activity in acetic acid rats was increased 18 fold in comparison to saline treated animals. However, by day 3, acetic acid-treated rats showed an approximate 30 fold increase in MPO activity over saline controls ($q_1 = 6.26$, $p < .01$) and the difference increased further on day 5 ($q_4 = 15.23$, $p < .01$). As with TNB animals, the colon of acetic-acid treated rats showed necrosis, edema and haemorrhage. There was no significant difference in level of inflammation between TNB and acetic acid-treated rats on any days tested ($q_2 = 2.88$, NS).



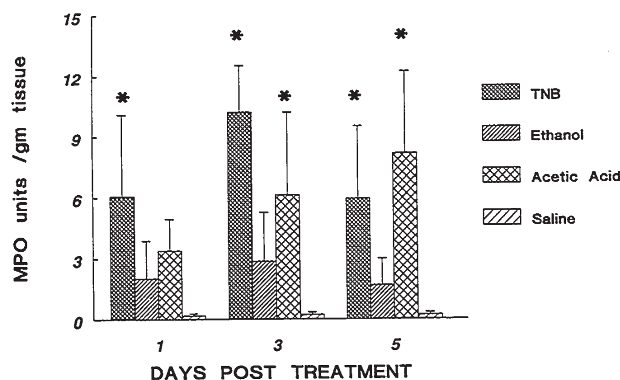



FIG. 1. Myeloperoxidase units per gram of wet tissue 1, 3, or 5 days following treatment with TNB (N = 9 on Days 1 and 3; N = 37 on Day 5), ethanol vehicle (N = 9 on Days 1 and 3; N = 39 on Day 5), acetic acid (N = 6 on Days 1 and 3; N = 16 on Day 5), or saline vehicle (N = 6 on Days 1 and 3; N = 17 on Day 5). Data shown are group means + 1 SE. Significant difference to respective control group indicated by *.

Changes in Food Intake and Body Weight

The effects of colonic inflammation on the intake of a solid powdered chow are shown in Figure 2. Administration of IR ethanol or saline did not reduce food intake relative to pre-treatment baselines. In contrast, both TNB and acetic acid treatments significantly suppressed food intake. Group differences in 24-hr food intake were significant on days 1, 2 and 3 post colitis: day 1: $F(3,156) = 287.7$, $p < .001$; day 2: $F(3,138) = 187.8$, $p < .001$; day 3: $F(3,133) = 56.16$, $p < .001$. The magnitude and time courses of food intake suppression induced by the two treatments were very similar: intake fell by 80%, 70%, and 50% on days 1, 2 and 3 following induction of colitis by TNB and by 80%, 71%, and 49% on the same days following induction of colitis by acetic acid.

Body weights, expressed as a percent of weight on the day prior to treatment, are shown in Figure 3. Control rats, treated with either IR ethanol or saline, gained a small amount of weight throughout the five day post-treatment period. In contrast, both TNB and acetic acid-treated groups lost weight within 24 hr after induction of inflammation and weights remained 10% less than controls on all five post-treatment days (p's< .05).



FIG. 2. Levels of 24-hr food intakes (powdered rat chow) in the baseline period and for five days following treatment with TNB (N = 50), ethanol vehicle (N = 58), acetic acid (N = 25), or saline vehicle (N = 27). Data shown are group means ± 1 SE. Significant difference to respective control group shown by *.

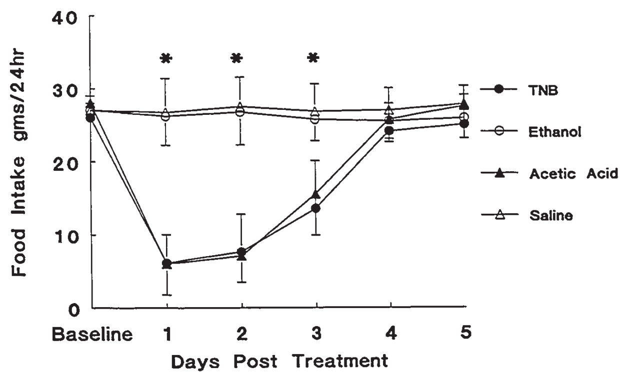
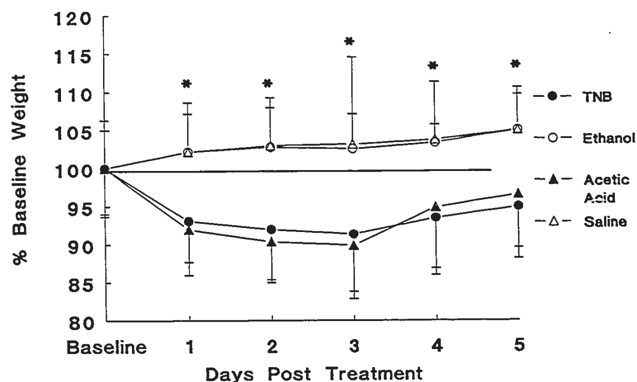





FIG. 3. Body weights (expressed as a percentage of pre-treatment weight) for five days following treatment with TNB (N = 50), ethanol vehicle (N = 58), acetic acid (N = 25), or saline vehicle (N = 27). Body weight on the day immediately before treatment represents 100%. Data shown are group means ± 1 SE. Significant difference to respective control group shown by *.






DISCUSSION

These results provide an initial characterization of changes in food intake and body weight in the rat model of experimental colitis induced by trinitrobenzene sulfonic acid and ethanol. The induction of colitis was confirmed by demonstrating significant increases in MPO activity in the colon during the first 5 days, as was found by others (84, 139). Any animals in which bowel perforations or peritonitis were found were excluded form the experiment. Colitis was accompanied by a substantial decrease in feeding that was maximum during the first 48 hrs and was reversible after 4 days. Changes in food intake were accompanied by a significant decrease in body weight. It remains to be determined whether all, or only part, of the weight loss can be attributed to the reduced food intake, for it is possible that increased catabolism could contribute to the weight loss. While necrosis of bowel tissue was noted, systemic inflammation due to peritonitis was not generally observed, any animals with perforation of the bowel were excluded from the experiments.

The suppression of feeding was not restricted to TNB-induced colitis since a reduction in feeding of similar magnitude and time-course was observed following the induction of colitis with acetic acid. These findings indicate that the anorexia does not depend on the agent used to induce inflammation. Taken in conjunction with previous demonstrations of decreased food intake in animal models of intestinal inflammation due to nematode infections (20, 130), these results suggest that the anorexia occurs as a result of the inflammatory response. The reliability of the TNB-induced colitis model, together with the present demonstration of clear cut and reproducible changes



in food intake, make this an eminently suitable model to further investigate the mechanisms underlying the ability of intestinal inflammation to alter feeding behaviour. The next series of experiments will attempt to show that TNB treated animals specifically decrease their food intake and do not show abnormalities in other feeding behaviours, or a "nonspecific malaise".

# CHAPTER 2.

## INTRODUCTION

In both humans and animals severe infection or inflammation often leads to a plethora of nonspecific symptoms including weakness, malaise, listlessness and an inability to concentrate (61). Additionally, hypersomnia, anorexia, loss of interest in usual activities are observed in infected humans or animals. These complex symptoms have been referred to as "sickness behaviour" in the context of organic illness (51). Sickness behaviour implies a more specific pathophysiology than is offered by the term "nonspecific malaise".

The severity of inflammation seen in TNB-treated rats demands that I demonstrate the observed anorexia to be a primary result of the inflammation and not secondary to other sickness behaviours. For example: a paralysed rat could be considered anorexic if one was only to measure total food intake. However, in this case, the paralysed rat would simply be incapable of performing the behaviour necessary to feed, and the anorexia would be secondary to its inability to move. In anorexia associated with TNB colitis, it is possible that rats: 1) are not physically capable of performing the behaviours necessary to feed, 2) are not specifically avoiding nutrients but, have stopped all ingestive behaviours, or 3) have formed an association between feeding and intestinal discomfort and thus have developed a conditioned taste aversion to the diet they are fed. By measuring water intake, maintaining the animals

on liquid and low residue elemental diets and using meal pattern analysis I have attempted to clarify these issues. For example, a normal level of water intake accompanied by a decreased intake of liquid diet would suggest that animals are specifically avoiding a nutritional load. As elemental diets are low residue, they produce very little fecal matter and should produce less mechanical stimulation of the inflamed colon, perhaps resulting in less discomfort (138). Thus the elemental diet would not cause an association in TNB treated rats between feeding and the movement of feces across the inflamed colonic segment. I also use meal pattern analyses to determine whether the suppression of food intake is associated with a reduction of meal size, meal number, or both. I use two conventional end of meal definitions (EOMD). The 40 min EOMD championed by La Magnen and the 10 min EOMD preferred by Castonguay (18). EOMD is the time elapsed after a meal that defines that meals termination as a distinct feeding event. Agents that reduce intake via malaise (eg. lithium chloride) do so primarily by decreasing the number of meals initiated (143). In contrast, agents believed to suppress eating by enhancing postprandial satiety do so predominantly by a reduction of meal size (18, 144). Thus, a specific reduction of meal size, with no reduction of meal frequency would further support our contention that colitic rats are not deficient in their ability to initiate or maintain appetitive behaviour. If an equal number of smaller meals is the result obtained, one might speculate that the anorexia results from a specific perturbation of meal controls, perhaps an exaggeration of a postprandial peripheral satiety mechanism. With these experiments I hope to show that TNB treated rats a) are capable of performing the behaviours



necessary to eat, b) do not show alteration of other ingestive behaviours c) do not have conditioned food aversions, d) specifically decrease meal size and not meal frequency.

MATERIALS AND METHODS

Measurements of Food and Water Intake

Food was available ad libitum and consisted of powdered rat chow (Purina # 5001), a nutritionally-adequate evaporated milk based liquid diet (64), or elemental diet (Peptamen, Clintec Nutrition , Mississauga, Ontario). Twenty-four hour food intakes were measured for seven consecutive days prior to, and for five days following TNB treatment. In one experiment, 24-hr water intakes were also measured over the same period.

Meal pattern analysis

Twelve rats were housed individually in metal cages with a food cup located in a 7 cm tunnel mounted on one end of the cage. Each food cup sat on a Sartorius analytic balance. A computer monitored the weight of the food cup every eight seconds. This apparatus, and the computer analysis program, have been described elsewhere (18, 59). Rats were maintained in this apparatus until a stable baseline of meal size and frequency had been established. Then, rats were treated with TNB (N=8) or the vehicle ethanol alone (N=4) and meal patterns were monitored for an additional four days.



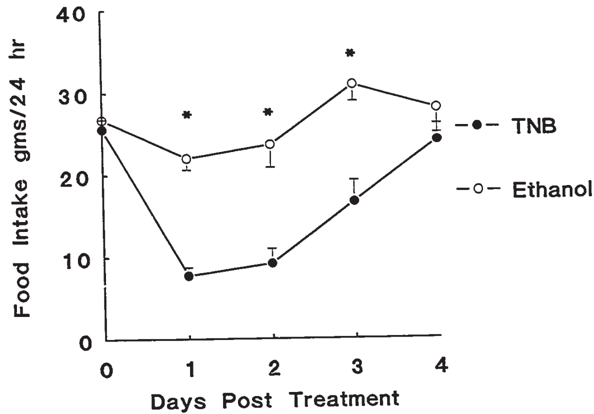
RESULTS

Meal pattern analysis

The mean weights of the eight animals constituting the TNB group and the four controls were similar at the time of treatment (286 ± 6 gms versus 275 ± 9 gms respectively). Also, the two groups had similar 24-hr food intakes prior to treatment (TNB: 26.0 ± 1.0 gms; controls: 25.5 ± 1.6 gms). As expected, rats treated with TNB ate significantly less than controls during the four post-treatment days, $F(1,10) = 79.88$, $p<.001$. The significant Group X Day interaction, $F(3,30) = 4.80$, $p<.01$, and inspection of Figure 4, indicates that TNB-treated rats were initially anorexic, but eventually recovered food intake. Multiple comparisons conducted with the Studentized Range statistic and evaluated according to the Newman-Keuls procedure, indicated that TNB-treated rats ate less than controls on Day 1, $q_4(30) = 8.82$, $p< .01$, Day 2, $q_4(30) = 8.69$, $p< .01$, and Day 3, $q_6(30) = 9.12$, $p< .01$. Total daily food intakes were not significantly different on Day 4, $q_2(30) = 2.29$, NS.



Figure 4. Daily (24-hr) food intakes in meal pattern study on the four days following induction of colitis (TNB, N=8) or control treatment (ETOH, N=4)). Day 0 represents intake in baseline period. Data shown are group means ± 1 SEM (where not shown, SEM falls within symbol). Significant group difference shown by *.






Meal pattern analyses are presented in Figures 5 and 6. Figure 5 presents the average meal frequency and size using a 10 minute end-of-meal definition (EOMD); Figure 6 uses a 40 minute EOMD. Meal frequency and size at each EOMD were analyzed with separate two-factor ANOVA's with Group as the between-group factor and Days as the within-group factor.

Using a 10 min EOMD, the two groups had similar meal frequencies, $t(10)= 2.03$, NS and sizes, $t(10) = 1.98$, NS, during the baseline period. Meal frequency was not significantly different between the two Groups, $F(1,10) = 1.90$, NS, nor did it change, $F(3,30) = 1.01$, NS, over the four post-treatment days. In contrast, rats treated with TNB displayed significantly reduced meal size following treatment, $F(1,10) = 10.0$, $p<.01$. Multiple comparisons indicated that TNB-treated rats had significantly reduced average meal size compared to controls on Day 1, $q_4(30) = 5.03$, $p< .01$ and Day 2, $q_4(30) = 4.59$, $p< .05$. Average meal size was still smaller on Day 3 but the difference just failed to meet statistical significance, $q_5(30) = 3.70$, $p< .08$. The average meal size of TNB and control groups did not significantly differ on Day 4, $q_3(30) = 2.54$, NS.

Similar results were obtained using a 40-min EOMD. Both groups had similar baseline average daily meal frequencies, $t(10) = 1.25$, NS, and sizes, $t(10) = .20$, NS. Meal frequency was not significantly different between the two Groups, $F(1,10) = 1.03$, NS, nor did it change, $F(3,30) = 1.28$, NS, over the four post-treatment days. However, the average meal size of the TNB-treated rats was significantly smaller, $F(1,10) = 9.88$, $p<.01$. Multiple comparisons indicated that rats treated with TNB ate smaller meals than controls on Day 1, $q_4(30) = 4.29$, $p<.05$, Day 2, $q_4(30) = 4.01$,

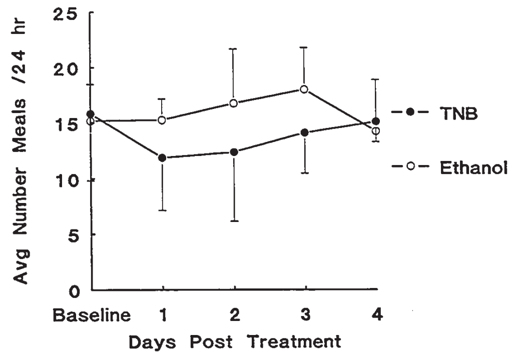


$p<.05$, and Day 3, $q_6(30) = 5.45$, $p<.01$. Average meal size for TNB-treated rats and controls was not significantly different by the fourth day following treatment, $q_2(30) = 2.19$, NS.

The specific reduction of meal size, and not meal frequency, was evident during both night- and day-time eating. For example, using a 10 min EOMD, there were no significant differences between TNB and control animals in meal frequency during the four post-treatment days in either day-time, $F(1,10) = 0.18$, NS, or night-time, $F(1,10) = 2.64$, NS. In contrast, meal size was significantly smaller in TNB-treated rats compared to controls in both day, $F(1,10) = 5.88$, $p < .05$, and night $F(1,10) = 8.60$, $P < .02$.



Figure 5. Average meal frequency (upper panel) and average meal size (lower panel) using a 10 min end-of-meal-definition on the four days following induction of colitis (TNB) or control treatment (ethanol). Day 0 represents values in baseline period. Data shown are group means ± 1 SEM. Significant group difference shown by *.



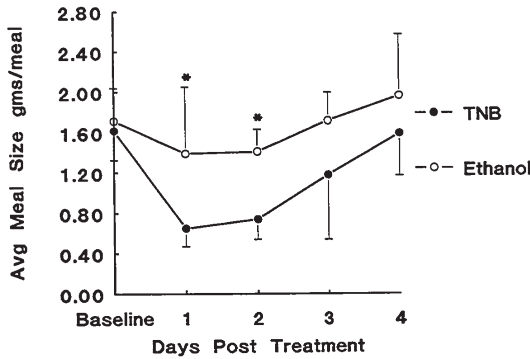
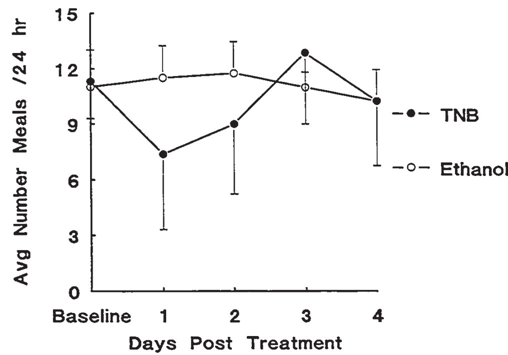
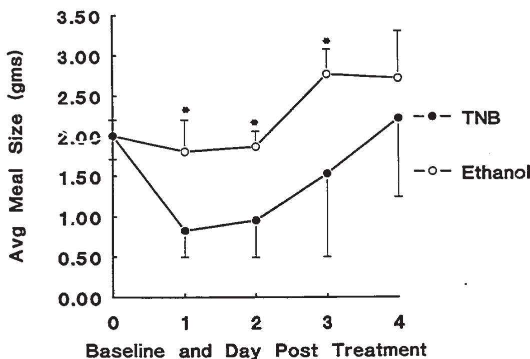




Figure 6. Average meal frequency (upper panel) and average meal size (lower panel) using a 40 min end-of-meal-definition on the four days following induction of colitis (TNB) or control treatment (ethanol). Day 0 represents values in baseline period. Data shown are group means ± 1 SEM. Significant group difference shown by *.






Alternate diets (liquid and elemental)

The suppression of feeding in TNB treated rats was not restricted to solid food. Figure 7 shows the 24-hr food intake of TNB treated rats maintained on a nutritionally-adequate liquid diet. The results paralleled the pattern of feeding observed in colitic rats maintained on solid food. The groups differed in level of food intake, $F(1,18) = 190.4$, $p < .001$, and the significant Group x Day interaction, $F(4,72) = 27.85$, $P < .001$, indicated the rats treated with TNB first decreased, but then recovered, food intake. The percentage decrease in food intake was 79% on both days 1 and 2 and 62% on day 3.

A similar pattern of anorexia was obtained when TNB treated rats were maintained on an elemental diet, as shown in Figure 8. Rats treated with TNB significantly suppressed elemental diet intake by 65% on day 1, and 40% on day 2.

Water intakes of TNB treated rats and controls for five days post-treatment days are shown in Figure 9. Control rats treated only with the ethanol vehicle showed no change in water intake compared to pre-treatment values. Rats treated with TNB drank significantly more water for five days post treatment than controls, $F(1,10) = 32.06$, $p < .001$.

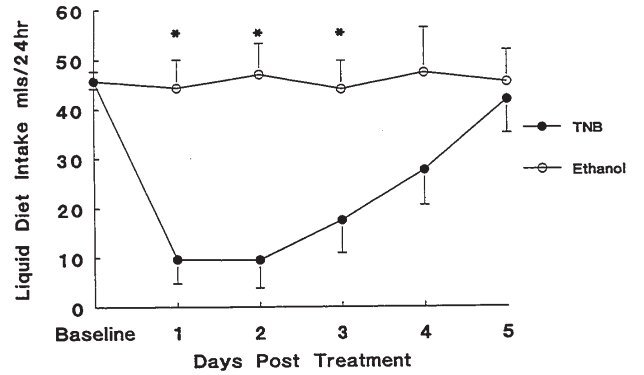



FIG. 7. 24-hr food intakes in the baseline period and for five days following treatment with TNB (N = 20) or ethanol vehicle (N = 20) in rats maintained on a nutritionally-adequate liquid diet. Data shown are group means ± 1 SE. Significant group difference shown by *.



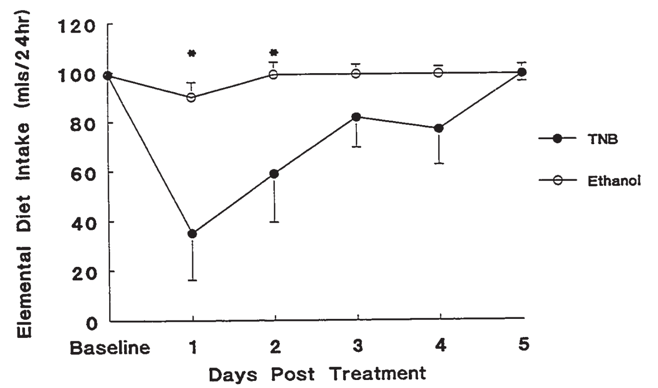
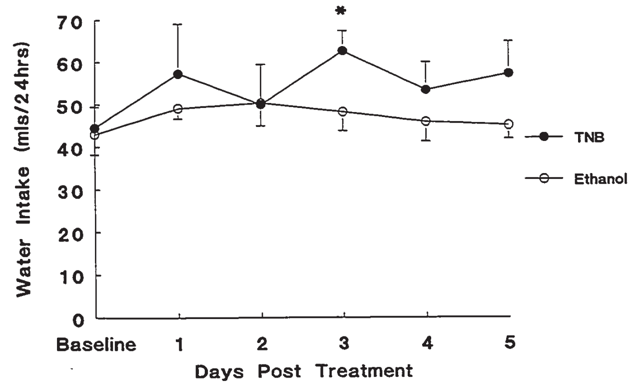



FIG. 8. 24-hr food intakes in the baseline period and for five days following treatment with TNB (N = 10) or ethanol vehicle (N = 10) in rats maintained on a low residue elemental diet. Data shown are group means ± 1 SE. Significant group difference shown by *.

FIG. 9. Twenty-four hr water intakes in the baseline period and for five days following treatment with TNB (N = 12) or ethanol vehicle (N = 12). Data shown are group means ± 1 SE. Significant group difference shown by *.






DISCUSSION

These experiments replicate the previously reported observations that IR infusion of TNB results, within 24 hrs, in a marked inflammation of the colon and that this inflammation is associated with an anorexia of approximately three days duration.

The current data also strongly reinforce the suggestion that the post-inflammation anorexia is not secondary to other sickness behaviours. I have demonstrated, using meal pattern analysis, that the reduction of eating results primarily from a suppression of meal size, with little change in the number of meals initiated. I demonstrated this using both a 40 min EOMD and a 10 min EOMD. These data again, are consistent with the our contention that malaise is an inadequate explanation for the reduction of eating in colitic rats. This profile of results is also consistent with the hypothesis that this pattern of meal pattern alterations are associated with abnormal post-prandial satiety signals and not a general malaise (143). Although, there was a trend toward decreased meal frequency it was not statistically significant. I recognise that the small sample size (particularly in the control group) could lead to a type II error. To avoid the type II error, the power of the analysis could be increased by enlarging the number of animals in the study. It is also possible however, that the highly sensitive measures used in meal pattern analysis led to a increased variability and in this way decreased power in the test.

Water intake is significantly elevated in TNB treated rats, resulting likely from the watery diarrhoea characteristic of this preparation. The suppression of feeding is manifest independent of the type of nutrient presented since there was a comparable



reduction in the intake of both solid, liquid and elemental diets. These results indicate that TNB-treated rats are motivated or at least capable of eating and show no generalized attenuation of appetitive behaviour. Finally, since MPO activity is still significantly elevated when feeding returns to normal, the ongoing inflammatory process (as indexed by MPO) alone is insufficient to impact on feeding. This suggests that discomfort due to the inflammatory process is an inadequate explanation for the observed anorexia. This is based on the assumption that any discomfort the rat was experiencing would be as severe on day 5 as day 1 as the inflammation is equally as large. In data not shown here changes in food intake were accompanied by a significant decrease in body weight in all cases. The data described in this chapter continue our attempts to characterize and validate an animal model to study the mechanisms by which inflammation of the gastrointestinal tract leads to the suppression of food intake.

Our data do not identify the specific mechanism by which colonic inflammation reduces eating. The profound anorexia, coupled with the meal pattern data, leave open the possibility that a reduced rate of stomach emptying resulting, perhaps, in a more rapidly developing gastric distention signal, accounts for the reduced meal size of colitic rats. Gastric emptying will be measured to determine if a delayed rate of emptying could be generating abnormal gastric satiety signals, contributing to the anorexia. The next series of experiments will use a sham feeding preparation, to examine the characteristics of how the anorexia is manifested.

## CHAPTER 3

INTRODUCTION

This chapter describes some relevant characteristics of the relationship between colonic inflammation, induced by IR infusion of TNB, and the resulting inhibition of eating. Understanding the behavioral repertoire associated with the anorexia may give insight into mechanisms underlying the anorexia. In the present studies, a sham feeding preparation is used to establish whether stimulation of post-gastric satiety mechanisms are required for the anorexia to fully manifest. Second, I evaluated the hypothesis that the reduction of eating associated with TNB colitis involves a reduced rate of gastric emptying. Specifically I wished to investigate the hypothesis that inflammation in one part of the gut (colon) can alter the function of a distant non-inflamed gut area (stomach). This would be significant especially if the function is altered in areas of the gut associated with production of satiety signals. This is based on a clinical study in patients with diagnosed ulcerative colitis, which does not affect the small intestine, who exhibit altered small bowel motility (73). This is further supported in animal studies where inflammation in one region of the gut changes contractility and neural function not only at the inflamed sight but also non-inflamed segments (58, 74). Thus, using a crude measure of gastric emptying I hope to delineate if the food intake changes seen in rats with TNB colitis are accompanied by delayed rate of gastric emptying.





MATERIALS AND METHODS

Sham feeding

Chronically indwelling stainless steel gastric cannulas were implanted into each of 16 rats. The cannula design and implantation procedure have been described elsewhere (140). Rats recovered for a minimum of 14 days following surgery. Then, rats were adapted to a 3-hr deprivation schedule: they were maintained on this deprivation schedule for the remainder of the experiment. Daily, rats were also trained to sham feed by having their cannula opened, being placed in test cages, and allowed to sham feed 1M sucrose for 30 minutes. The sham feeding cages and the procedure to prepare rats for sham feeding have been described previously (141). After 30-min sham intakes had stabilized, half the rats were treated with TNB; the other half with ethanol alone. Sham feeding continued from the day following treatment for five additional days. For a five day baseline period, and on all post-treatment days, the amount of powdered chow eaten in the home cage by the rats during the 20.5 hrs until food deprivation was imposed again was also measured.

After the last sham feeding session, rats were stunned by rapid deceleration and sacrificed by cervical dislocation. Approximately 2 cm of descending colon was removed for myeloperoxidase (MPO) assay, a measure of the degree of tissue inflammation (122), according to the procedure described previously.

Gastric emptying analysis

Twenty four rats were divided in equal numbers into four groups so that the



24-hr food intakes of the groups would be equal. Half the animals were treated with TNB, the other half with ethanol alone. Half the animals would be sacrificed 60 min post-gavage and half 90 min post-gavage. Animals were deprived of food for 24-hr after treatment. After the deprivation period, all rats received a 15 ml high fat liquid diet gavage (64). Then, either 60 or 90 min post-gavage, rats were lethally anaesthetized with a 1 ml intraperitoneal injection of sodium pentobarbital (Somnotol). Stomachs were ligated with 3-0 silk at the lower esophageal and pyloric sphincters. Stomachs contents were removed, dried and then weighed to determine residual gastric contents. Half the animals in each treatment group were sacrificed at the 60 or 90 min time point. The percent of the original 15 ml load remaining in the stomach was determined by dividing the dry weight of the stomach contents by the mean dry weight of three 15 mls liquid diet test dishes. Also, colon samples were collected and assayed for MPO activity as described previously.

RESULTS

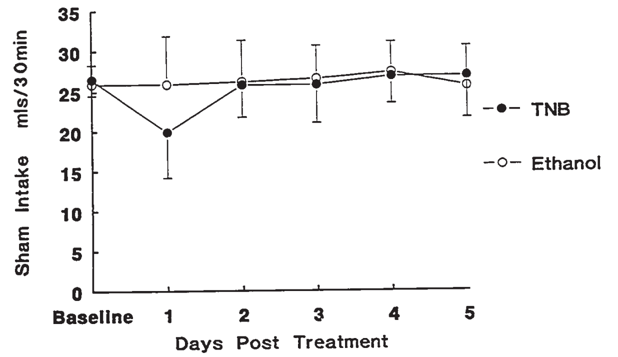
Sham feeding

TNB and control rats were equivalent in body weight (TNB: 342 ± 5 gms; controls: 345 ± 6 gms), sham intake (TNB: 26.1 ± 2.9 gms; controls: 26.3 ± 2.2 gms) and 24-hr food intake (TNB: 25.8 ± 1.4 gms; controls: 25.8 ± 1.5 gms) prior to treatment. The sham and real feeding intakes for five days post-treatment are shown in Figure 10. ANOVA revealed that TNB treatment did not affect the amount sham



fed, $F(1,14) = 1.50$, NS. An *a priori* t-test comparing group differences even on the first day post-treatment revealed no significant group differences, $t(14) = 2.00$, NS. In contrast, these same TNB-treated rats, that demonstrated no reduction of sham feeding, ate significantly less than controls in the remaining 20.5 hrs of ad lib intake in the home cage, $F(1,14) = 43.01$, $p < .0001$.

MPO analyses five days post treatment confirmed a colitis in rats treated with TNB. The group average MPO activity five days following TNB treatment was 9.26 ± .6 U/g for TNB-treated rats and 1.76 ± .2 U/g for vehicle-treated animals. This difference was statistically significant, $t(14) = 11.9$, $p < .001$.



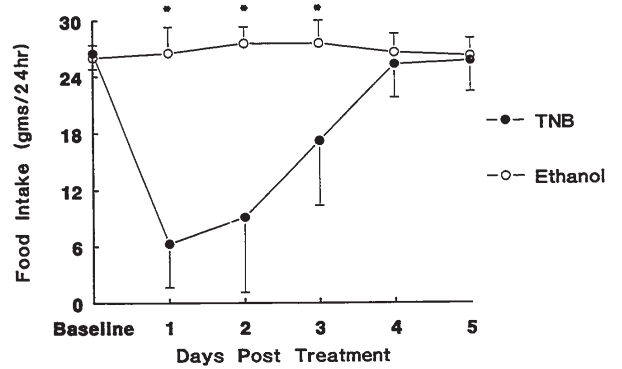




FIG. 10. Average amount sham fed in 30-min (upper graph) and amount real fed in the remaining 20.5 hr in the home cage (lower graph) for TNB(n=8) or ethanol-treated (n=8) rats in baseline period (Day 0) and the five days after treatment. Numbers represent group mean + 1 SEM. Significant group difference shown by *.



Gastric emptying

The results of the gastric emptying tests are presented in Figure 11. Differences between the TNB and control groups at each of the two time points were assessed with a two-factor ANOVA. Results indicated that TNB-treated rats emptied significantly slower than their controls, $F(1,20) = 24.69$, $p<.001$. Multiple comparisons revealed that the slowed emptying by TNB rats relative to controls was apparent at both the 60 minute, $q_3(20) = 3.79$, $p<.05$, and 90 minute, $q_3(20) = 6.12$, $p<.01$, time points. MPO levels for the four groups of animals are presented in Table 1. MPO levels were already significantly elevated in rats treated with TNB, $t(22) = 5.96$, $p<.001$, even though tissue was assayed only 24-hr following treatment



Figure 11. Percentage of total gavaged gastric content remaining in the stomach at times indicated following direct intragastric infusion of a test meal at time 0. Data shown are group means ± 1 SEM (N=6 in each group). Significant group difference TNB vs Ethanol shown by *.

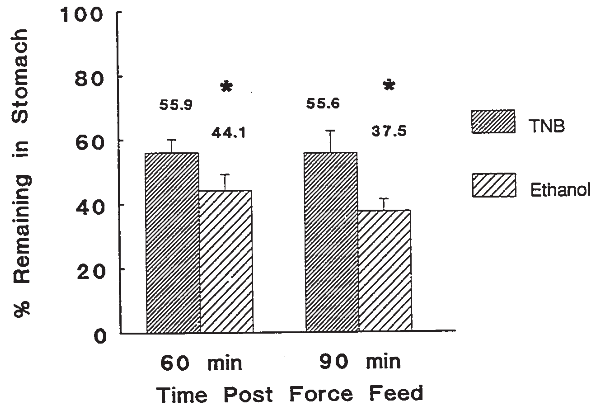





Table 1. Colon MPO values for four groups of rats in gastric emptying study. Groups are indicated by the type of treatment received, trinitrobenzene sulfonic acid (TNB) or the ethanol vehicle alone, and the amount of time the test load was permitted to empty from the stomach, 60 or 90 min. Samples were obtained immediately after the gastric emptying test, 24-hr after treatment with either TNB or EtOH. One myeloperoxidase (MPO) unit is equal to the amount of MPO required to convert 1 μmol of $H_2O_2$ to $H_2O$ in 1 min at 27 C. Significant group difference TNB vs Ethanol shown by *.

| Group | Emptying Time | *n* | MPO (U/g) |
|---|---|---|---|
| TNB | 60 | 6 | 6.5±0.7* |
| | 90 | 6 | 4.2±0.8* |
| Ethanol | 60 | 6 | 1.6±0.5 |
| | 90 | 6 | 0.9±0.1 |



DISCUSSION

This study was designed to characterise more completely the reduction of eating associated with experimental colitis in the rat. I again, replicated my previously reported observations that IR infusion of TNB results, within 24-hr, in a marked inflammation of the colon and that this inflammation is associated with an anorexia of approximately three days duration. In colitic rats, sham feeding levels are unperturbed even on days following induction of colitis when ad lib real feeding is greatly reduced. In light of the previously reported water intake, the sham feeding data further supports the contention that the animals are capable of performing the behaviours required to feed both liquid and elemental diets.

Gastric emptying is significantly slowed in TNB treated animals. While it would be convenient to ascribe causality for the anorexia to increased gastric satiety signals due to slowed emptying it is unwise to do so. It is well accepted that gastric distention per se induces meal termination only at the upper limits of stomach stretch (27). Therefore while a contributing role of delayed gastric emptying can not be ruled out, it is unlikely to be the sole cause.

At present, it is unclear which anorexigenic signals produced by the inflamed segment inhibit eating and whether these signals communicate with relevant brain sites directly (e.g., via endocrine mechanisms) or indirectly (e.g., via their influence on other peripheral or central signals). Tumour necrosis factor and the interleukins are established mediators of inflammation-induced anorexia (10, 11, 54, 61, 78, 82, 86, 87, 90, 92, 94, 98, 111, 125) and are elevated in cases of inflammatory bowel disease



(45, 70, 128). It has been speculated that these cytokines inhibit eating via their effects on gastric emptying, similar to my observations in TNB treated rats (94, 110). Alternatively, the presence of interleukin receptors in multiple sites in brain (50), particularly in regions associated with the control of eating, and the recent description of an IL-1 transport mechanism across the blood-brain barrier (5), leave open the possibility that the central nervous system detects anorexigenic signals produced by the inflamed segment directly. This is at least circumstantial evidence that cytokines (particularly IL-1) might be involved in the anorexia displayed by TNB treated rats. The next chapter will use inhibitors of arachidonic acid metabolism and an interleukin-1 receptor antagonist to investigate the role these inflammatory products play in the TNB induced anorexia.

CHAPTER 4

INTRODUCTION

As described in Chapter 1, inflammation of the colon induced by intra-rectal administration of TNB, results in a large, but transient, anorexia expressed as a dramatic reduction of daily food intake. The preceding two chapters suggest that malaise resulting from the tissue injury is inadequate to explain the inhibition of feeding following TNB treatment. Rather, it seems the anorexia depends upon the elaboration of an inflammation-related signal that is communicated to the brain. Thus, the anorexia could be a result of an abnormal satiety signal reaching the brain or a signal to the brain which causes normal satiety signals to be sensed as abnormal. The objective of the present chapter is to evaluate the contribution of several such putative anorexigenic signals, the arachidonic acid metabolites: prostaglandin $E_2$ ($PGE_2$) and leukotriene $B_4$, ($LTB_4$) and the cytokine interleukin-1 (IL-1).

The general introduction described the effects of some cytokines on areas of the gastrointestinal tract involved in the control of feeding including the stomach and duodenum. Here, as an initial exploration of which anorexic signals could be generated peripherally, I examined the role of arachidonic acid metabolites in the feeding changes. Products of arachidonic acid metabolism, are found in increased concentration in the TNB-induced colitis model (12, 14, 36, 139). Specifically, leukotrienes (the lipoxygenase products of arachidonic acid) have been demonstrated to be elevated in





the tissue of animals treated with TNB (14, 104, 139). Leukotrienes are considered to be mediators of the inflammatory process in this model since inhibition of leukotriene synthesis has been shown to accelerate the rate of bowel healing in TNB colitis (139). Therefore, I investigated whether inhibition of leukotriene synthesis with a specific 5' lipoxygenase blocker (96) could alter the profile of anorexia seen in rats with TNB colitis.

The cyclooxygenase products of arachidonic acid metabolism (prostaglandins) have also been shown to be elevated in the tissue of rats treated with TNB (12, 14, 139). Treatment with prostaglandin analogues has been demonstrated to be mucosally protective in TNB colitis (3). There is also a model of intestinal inflammation induced by inhibition of cyclooxygenase that is not expressed in fasting animals suggesting a link between prostaglandins, inflammation and feeding (116). These findings further suggest a protective role for prostaglandins in the intestine. However, inhibition of cyclooxygenase products with indomethacin can abolish the anorexia observed with the administration of exogenous IL-1, another putative mediator of the anorexia in this model (134). Here, I used the cyclooxygenase inhibitor indomethacin to prevent prostaglandin synthesis in an effort to evaluate the role of endogenous prostaglandins in the anorexia I observe with TNB colitis.

The polypeptide cytokine IL-1 is a product of activated macrophages that is important in the initiation and maintenance of the inflammatory process (29, 30). Several observations implicate IL-1 in the anorexia following colon inflammation. First, a suppression of feeding is observed within the first 24-hr following TNB



treatment and IL-1 is the only cytokine elevated in the colon within first 24-hr after TNB administration (104). Second, acute administration of IL-1, peripherally or centrally, suppresses eating in rats (29, 30, 54, 78, 81, 98, 99, 100). More importantly, chronic peripheral administration of IL-1 results in a time course of anorexia identical to that produced by TNB treatment (86, 92) and IL-1 is a potent stimulator of prostaglandin production (60). Third, the inhibition of eating associated with acute or chronic IL-1 administration is attenuated by inhibition of cyclooxygenase activity (54, 82, 134).

IL-1 receptors are present in both the periphery and brain (29, 30, 50, 111). In other studies, the anorexic effects of peripherally- or centrally-administered IL-1 (61, 98, 99), as well as inflammation of skin or gastrointestinal tract (23, 39), have been blocked by administration of a recombinant human IL-1 receptor antagonist (rhIL-1ra) (34). In this chapter, I use rhIL-1ra administered either peripherally (subcutaneously-SC) or centrally (intracerebroventricularly-ICV) to evaluate the contribution of peripheral and central IL-1 receptors in the inhibition of eating following colon inflammation induced by TNB. Thus, in this chapter I hope to identify the possible signals that are released in the periphery (possibly at the site of inflammation) and are able to communicate an anorexigenic signal to the brain. The hypothesis to be tested in this chapter is if the suppression of feeding observed in rats with TNB induced colitis is due to the inflammatory process in the colon.



MATERIAL AND METHODS

Manipulation of Arachidonic Acid Metabolism

To inhibit leukotriene synthesis, 10 mg/kg of the 5' lipoxygenase inhibitor MK-886 (41), suspended in carboxymethylcellulose (Merck-Frosst, Canada) was given by IR injection 24-hr and 2-hr before, and 22, 46, 70, and 96-hr after TNB. This dose was selected because of its proven efficacy in decreasing leukotriene production (79), and accelerating the rate of bowel healing in rats with TNB colitis (139). Ethanol control received MK-886 using the same dose schedule before and after IR administration of ethanol. Control infusions of carboxymethylcellulose were also administered to both TNB and ethanol animals. The efficacy of MK-886 in inhibiting leukotriene synthesis, was assessed by radioimmunoassay of LTB4 in tissue from the distal colon as described elsewhere (139).

Indomethacin (Sigma, St.Louis, MO.), a potent inhibitor of cyclooxygenase activity (107) was dissolved in 7% sodium bicarbonate vehicle (5mg/ml) and administered by IP injection 24 hrs and 2 hrs before, and 22hrs, 46hrs, 70hrs and 96 hrs following induction of colitis by TNB. Each rat received indomethacin in a dose of 5 mg/kg (135). The efficacy of indomethacin treatment was evaluated by radioimmunoassay for PGE2 in tissue from the distal colon as described elsewhere (139).

Receptor Antagonist Delivery

Osmotic mini-pumps (Alzet model 2001, Alza Corporation) were loaded with

either rhIL-1ra dissolved in 0.15M saline or 0.15M saline alone and placed in a 37° saline bath for 4 hrs to allow pumps to reach a steady rate of delivery. For peripheral delivery, pumps were loaded with either 6 or 10 mg of rhIL-1ra in 250ml of 0.15M saline resulting in a delivery of 24 µg/hr or 40 µg/hr respectively. For central infusion, pumps were loaded with 6 mg rhIL-1ra in 250ml 0.15M saline, to deliver 24 µg/hr.

For peripheral administration, animals were lightly anaesthetized with ether. Pumps were implanted SC in the scapular region and the incision was closed with wound clips. For central administration, rats were anaesthetized with sodium pentobarbitol (65 mg/kg) and, using standard stereotaxic procedures, a 28 ga stainless steel cannula was implanted into the lateral cerebral ventricle (1.8 mm posterior and 0.2 mm lateral to bregma, 3.5 mm below the skull). The cannula was embedded in a head-cap constructed of dental acrylic and the entire assembly was secured to the skull with four stainless steel screws. The cannula was connected to the osmotic mini pump. Pumps were weighed before implantation and at the end of the experiment to ensure complete delivery of their contents. For central infusions, cannula placements were also verified at the end of the experiment by infusing a neutral red solution through the catheter, removing the brain, sectioning and observing the location of the stain.

Determination of serum IL-1ra Levels

At sacrifice, blood was withdrawn by cardiac puncture into heparinized tubes, centrifuged, and the plasma stored at -70°. Levels of rhIL-1ra were determined using a capture ELISA. At the lowest dilution (1:5), the lower detection limit of the assay



was 3.9 ng/ml.

Statistical analysis and abbreviations

Results were analyzed with analysis of variance (ANOVA). Where warranted ($p<.05$) multiple comparisons were performed using the Studentized range statistic (q) and evaluated according to the Newman-Keuls procedure. A probability level of less than 0.05 was accepted as the significance level. For clarity, the treatment and control groups in the IL-1ra experiment are indicated by the following acronym: "X/Yz", where "X" indicates either IR TNB or EtOH, "Y" indicates the delivery of rhIL-1ra (ra) or .15M saline (sal) and "z" indicates whether the rhIL-1ra or .15M saline were delivered centrally (c) or peripherally (p). For example, TNB/ra-c indicates a group of rats treated with IR TNB and receiving rhIL-1ra injected ICV.

## RESULTS

Effect of MK-886.

TNB caused a 2-fold increase in LTB-4 in the colon and this increase was prevented by administration of MK-886 (Table 2). Table 2 also shows that TNB significantly increased MPO activity $F(3,12) = 8.83$, $p< .01$, and that treatment with MK-886 had no effect on the degree of tissue inflammation ($F(3,12) = 8.83$, $p< .01$). The effect of MK-886 on food intake is shown in Figure 12. Over the five days post-treatment, TNB-treated groups differed significantly in the level of food intake from controls



($F(2,9) = 25.91$, $p< .001$), and multiple comparisons revealed that the pattern of intake was similar in colitic rats regardless of whether they had received MK-886.



Table 2. Tissue LTB-4 (pg/mg tissue) and MPO levels. Values indicated are X ± SEM. MK-886 delivered at a dose of 10 mg/kg. Significant group difference TNB vs respective Ethanol control shown by *.

| Treatment | LTB-4 | MPO |
|---|---|---|
| IR ethanol + saline | 150 + 45 | 2.13 ± .4 |
| IR ethanol + MK-886 | 152 ± 33 | 2.23 ± .4 |
| IR TNB + saline | 378 ± 100* | 11.13 ± 2.5* |
| IR TNB + MK-886 | 124 ± 16 | 10.03 ± 2.0* |

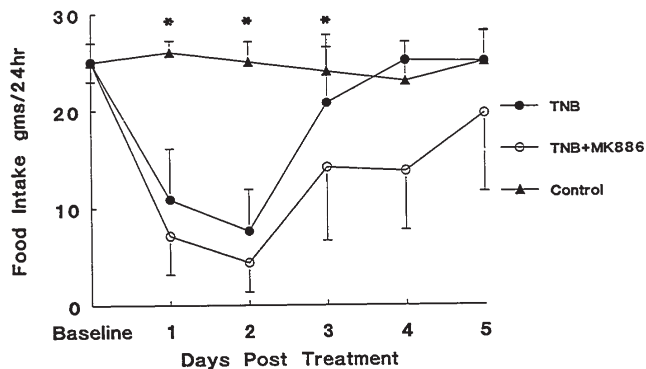



FIG. 12. Twenty-four hr food intakes (powdered chow) during the baseline period and for five days following TNB (N = 4), TNB plus leukotriene synthesis inhibitor MK-886 (10mg/kg) (N = 8) or controls (pooled data for ethanol vehicle (N = 4) and ethanol plus MMK-886 (N = 4). Data shown are group means + 1 SE. Significant group difference for both TNB+MK886 and TNB alone compared to controls shown by *.



Effect of Indomethacin.

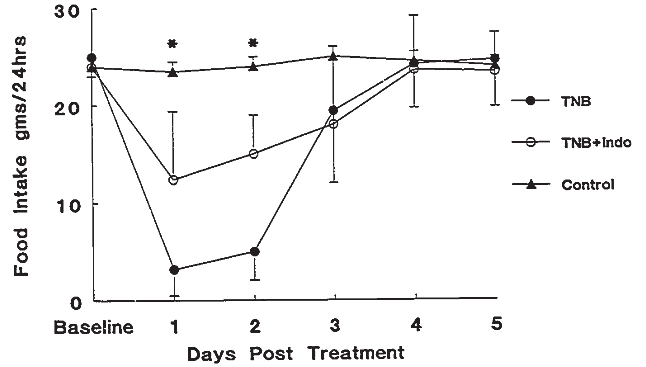
Administration of TNB produced a seven-fold elevation of PGE-2 in the colon compared to ethanol controls ($P < .001$). Indomethacin treatment reduced PGE-2 levels to control values in TNB-treated rats but had no effect on the level of tissue inflammation induced by MPO activity (Table 2). The effect of indomethacin treatment on food intake is shown in Figure 13. Multiple comparisons revealed that, on both Days 1 and 2, controls ate significantly more than TNB or TNB + indomethacin rats ($p$'s< .01). More importantly, however, rats treated with TNB + indomethacin ate over three times more than rats treated with TNB alone on both day 1 ($q_2 = 5.50$, $p < .01$) and day 2 ($q_2 = 5.39$, $p < .05$). By day 3 all rats had recovered to similar levels of food intake.



Table 3. Tissue PGE-2 (ng/mg tissue) and MPO levels. Values indicated are $\bar{X}$ ± SEM. Indomethacin administered at a dose of 0.5 mg/kg. Significant group difference TNB vs respective Ethanol control shown by *.

| Treatment | PGE-2 | MPO |
|---|---|---|
| IR ETOH + saline | 1.8 ± .3 | 1.87 ± .7 |
| IR ETOH + Indo | 1.7 ± .3 | 2.02 ± .6 |
| IR TNB + saline | 12.8 ± 4* | 11.1 ± 1.7* |
| IR TNB + Indo | 1.5 + 4.8 | 9.2 ± 3.1* |

FIG. 13. Baseline and five day post treatment food intake for powdered rat chow following IR TNB plus IP saline (N = 5), IR TNB plus IP indomethacin (.5mg/kg) N = 4) or their respective controls IR ethanol with or without indomethacin expressed together (N = 9). Significant group difference between TNB+indo and TNB alone shown by *.






Effect of Peripheral 40 µg/hr IL-1ra

Figure 14 shows the effects of peripherally-administered 40µg/hr rhIL-1ra on daily food intake following IR TNB or EtOH treatment. EtOH IR did not affect food intake compared to baseline regardless of whether it was paired with peripheral rh1L-1ra or saline. As expected on the basis of previous studies, IR TNB accompanied by peripheral saline significantly reduced food intake compared to EtOH controls on day 1 ($q_6$ (88)= 14.07, p< .01), day 2 ($q_7$ (88)= 15.83, p< .01), and day 3 ($q_{11}$ (88)= 10.24, p<.01). By day 4, intake of TNB rats receiving saline returned to normal. Peripheral administration of 40 µg/hr rh1L-1ra attenuated the magnitude of the anorexia induced by TNB. Specifically, although food intake of TNB rats receiving peripheral rhIL-1ra was significantly less than EtOH treated rats receiving rhIL-1ra, on day 1 ($q_{14}$ (88)= 10.44, p< .01) and day 2 ($q_{14}$ (88)= 7.14, p< .01). TNB rats treated with rhIL-1ra ate significantly more than TNB and saline animals on day 1 ($q_5$ (88)= 7.94, p< .01), day 2 ($q_4$ (88)= 11.46, p< .01), and day 3 ($q_{10}$ (88)= 10.14, p< .01).

Changes in food intake were paralleled by changes in body weight, shown in Figure 15. Animals treated with IR EtOH gained weight over the five post-treatment days. TNB treatment was associated with significant weight loss. However, TNB-treated rats receiving rhIL-1ra lost less weight than TNB rats receiving saline and, in fact, gained some weight over the post-treatment period.

Figure 16 and statistical analyses show that TNB treatment significantly elevated MPO levels compared to EtOH ($F_{1,21}$=103.3, p<.0001); peripheral rh1L-1ra had no effect on the degree of tissue inflammation produced by either TNB or EtOH.



Fig. 14. Twenty-four hour food intakes before treatment (Day 0) and for five days after treatment with TNB [(TNB/sal-p, n=8), (TNB/ra-p, n=7)], or EtOH [(EtOH/sal-p, n=8), (EtOH/ra-p, n=3)]. Data shown are group means ± SE. Significant group difference between TNB/ra-p and TNB/sal-p shown by *.

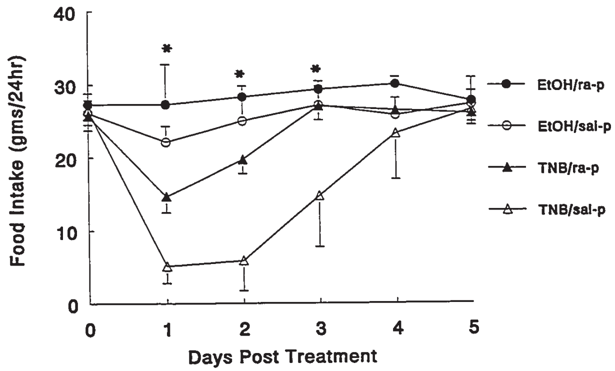



Fig. 15. Body weight changes over the five days following treatment with TNB or EtOH. Group N's given in legend of Fig. 1. Body weight on day immediately before TNB or EtOH treatment (Day 0) represents 100%. Data are group means ± SE.



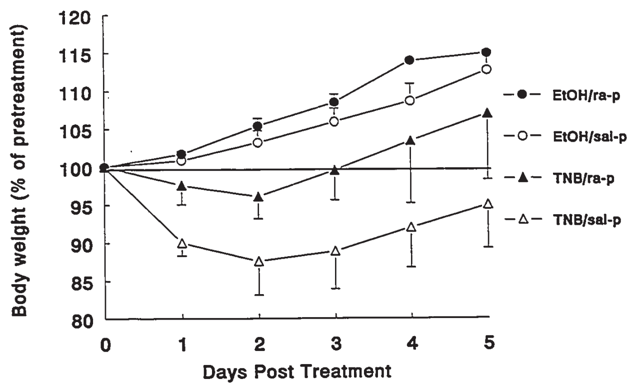
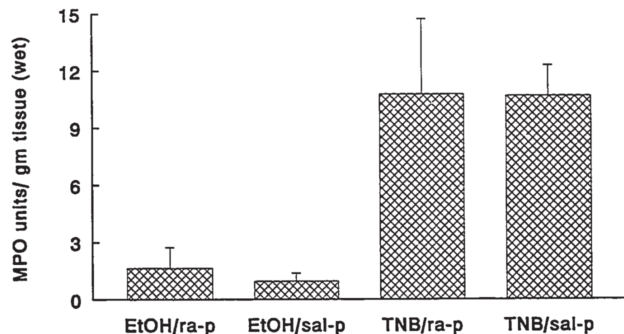



Fig. 16. Myeloperoxidase (MPO) activity in colon on day five following treatment with TNB or EtOH. Group N's provided in Fig. 14. Data are group means ± SE. Significant group difference shown by *.






Effects of Central and Peripheral (24 µg/hr) rh IL-I ra

The effects of 24µg/hr rh1L-1ra on the daily intake of powdered chow following colon inflammation are shown in Figure 17. EtOH IR did not affect food intake regardless of whether it was accompanied by central administration of rhIL-1ra or saline. Similarly, central administration of rhIL-1ra by itself had no effect on food intake in EtOH treated animals. As expected, IR TNB paired with ICV saline reproduced a time course of reduced food intake seen in previous studies. Specifically TNB and central saline rats ate less than EtOH and central saline on day 1 ($q_7(60)=13.6$, $p<.01$), day 2 ($p_{21}(60)=18.1$, $p<.01$), and day 3 ($q_{11}(60)=13.6$, $p<.01$). Rats receiving TNB and central rhIL-1ra also had significantly decreased food intakes compared to their controls, EtOH and central rhIL-1ra, but only on day 1 ($q_6(60)=6.2$, $p<.01$) and day 2 ($q_{19}(60)=8.22$, $p<.01$). More importantly, rats receiving central rhIL1-ra with TNB ate significantly more than rats treated with TNB but receiving central saline on day 1 ($q_3(60)=7.27$, $p<.01$), day 2 ($q_5(60)=10.4$, $p<.01$), and day 3 ($q_{10}(60)=5.62$, $p<.01$). I also administered the 24 µg/hr dose of rhIL-1ra peripherally. As shown in Figure 17, and in contrast to the effects of the central infusion, this dose of rh1L-1ra infused peripherally did not affect the magnitude or time course of anorexia induced by TNB.

The effects of colitis, with and without infusion of 24µg/hr rhIL-1ra, on body weight are shown in Figure 18. Both groups receiving IR EtOH increased body weight slightly over the five post-treatment days. Administration of TNB in conjunction with saline ICV or rhIL-1ra peripherally resulted in similar amounts of weight loss. In



contrast, TNB administered with rhIL-1ra ICV resulted in only a small initial decrease in body weight and, ultimately, in weight gain compared to pretreatment levels. Group mean MPO's are shown in Figure 19. Treatment with TNB significantly increased MPO activity compared to EtOH controls ($F_{1,13}=155.12$, $p<.0001$). MPO levels were unaffected by concomitant central or peripheral infusion of 24µg/hr rh!L-1ra in TNB and EtOH treated animals.



Fig. 17. Twenty-four hour food intakes before treatment (Day 0) and for five days after treatment with TNB [(TNB/sal-c, n=4), (TNB/ra-c, n=6), (TNB/ra-p, n=3)], or EtOH [EtOH/sal-c, n=4), (EtOH/ra-c, n=3)]. Data are group means ± SE. Significant group difference TNB/ra-c vs TNB/sal-c shown by *.

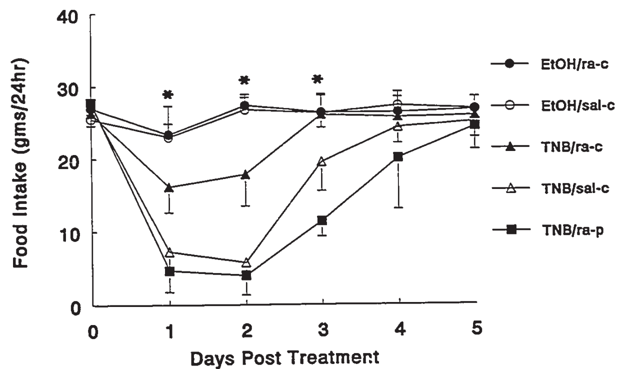



Fig. 18. Body weight changes over the five days following treatment with TNB or EtOH. Group N's as in legend to Fig. 17. Body weight on day immediately before TNB or EtOH treatment (Day 0) represents 100%. Data are group means ± SE.



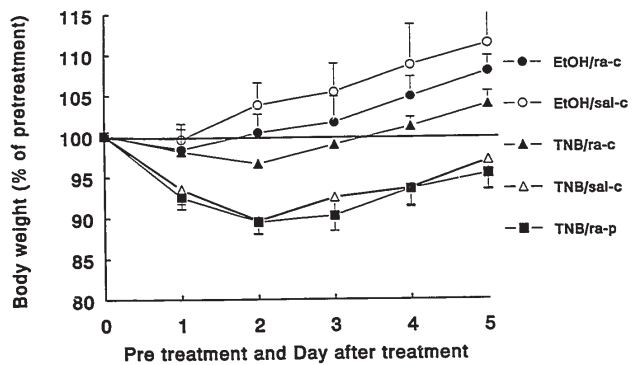
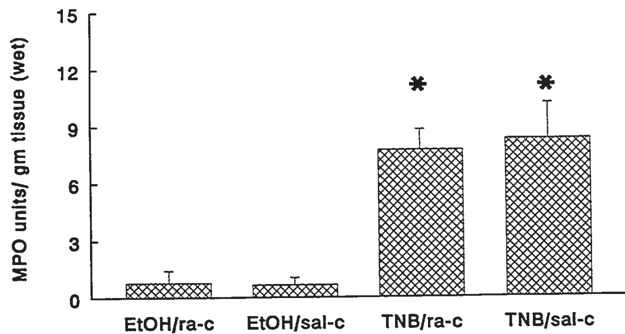



Fig. 19. Myeloperoxidase (MPO) activity in colon on day five following treatment with TNB or EtOH. Group N's as in legend to Fig. 17. Data are group means ± SE. Significant group difference shown by *.



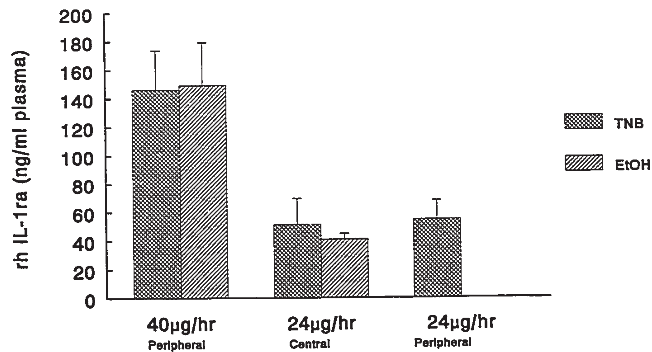



IL-1ra serum levels

Based upon measured differences in their initial and final weights, all pumps had delivered at least 90% of their contents during the course of the experiment. In addition, all rats with ICV cannulas had confirmed placements in the lateral cerebral ventricles based upon the localization of neutral red to the ventricle. All animals receiving saline via mini-pumps, either SC or ICV, had rhIL-1ra levels < 3.9 ng/ml. Plasma rhIL-1ra levels for animals receiving either SC or ICV rhIL-1ra are shown in Figure 20. Peripheral infusion of 40 μg/hr rhIL-1ra resulted in plasma levels nearly double those achieved by the 24μg/hr infusion (SC or ICV). There were no differences in the plasma rhIL1-ra levels achieved by identical doses in TNB- or EtOH-treated animals.



Fig. 20. Serum rhIL-1ra levels (ng/ml plasma) from rats treated with TNB or EtOH infused peripherally or centrally with rhIL-1ra at doses shown.



DISCUSSION

This series of experiments demonstrates that leukotrienes are likely not involved in the anorexia induced by TNB. Although MK886 inhibited leukotriene synthesis in this model, it did not affect MPO activity or the pattern of feeding. Our results with MK-886 differ from previously reported results demonstrating a decrease in MPO activity in rat colon when treated with this drug before and after TNB. The lack of effect in our experiments may reflect the fact that I looked at MPO activity only 5 days post TNB and that previous reports examine time points approximately one week later (139). This would allow for a further one week of treatment with MK-886, and may explain the conflicting results.

The results with indomethacin strongly indicate that production of prostaglandins is an essential step in the full expression of anorexia in TNB treated rats. The data with indomethacin treatment also argues against the malaise hypothesis. This is the case, as indomethacin attenuates the reduction of eating observed in TNB treated rats, although the level of colon damage in those animals, reflected by MPO activity, was as severe as TNB-treated rats without indomethacin. This suggests that animals do not increase their food intake because indomethacin is decreasing their pain or discomfort, assuming of course, a similar level of MPO activity reflects a comparable degree of discomfort due to the inflammation. There is also the implicit assumption that indomethacin effects are due to the down-regulation of cyclooxygenase products and not due to other non-specific effects.

This study was also designed to evaluate the contribution of IL-1 to the anorexia



and weight loss following the induction of colitis by administration of TNB in the rat. Our results indicate that peripheral delivery of 40mg/hr rhIL-1ra significantly attenuates the anorexia and weight loss seen in the TNB model. This finding is consistent with previous reports that peripherally injected rhIL-1ra results in a partial reversal of the anorexia induced by injection of exogenous IL-1 (10, 61, 111). Our results also parallel other findings showing that repeated peripheral injections of murine IL-1ra partially inhibit the anorexia and weight loss induced by subcutaneous skin tumours in mice (39).

Our experiment providing peripheral or central administration of 24µg/hr rhIL-1ra is more conclusive with regard to the location where the rhIL-1ra is working. The results indicate that this dose of rhIL-1ra: 1)does not increase feeding in EtOH treated rats, indicating central IL-1 receptors are not involved in the "normal" control of food intake 2)significantly attenuates the food intake suppression in TNB treated rats when administered ICV and 3) has no effect on the anorexia when delivered peripherally. The latter two results implicate central IL-1 receptors in the mediation of TNB induced anorexia. Our data does not allow us to rule out completely an involvement of peripheral IL-1 receptors. However, an alternative interpretation of the effectiveness of 40mg/hr rhIL-1ra administered peripherally, coupled with a lack of effect of 24mg/hr rhIL-1ra infused peripherally, is that only the higher infusion level achieves rhIL-1ra levels in the brain sufficient to antagonize IL-1 receptors. A direct test of this hypothesis requires measurement of rhIL-1ra levels in brain cerebrospinal fluid (CSF) after peripheral infusion.

GENERAL DISCUSSION

The idea that intestinal inflammation is associated with anorexia is derived from clinical observations in patients with active inflammatory bowel disease and from a limited number of studies in animals with intestinal inflammation secondary to infection by enteric parasites. (1, 53, 76, 112). The availability of simpler models of inflammation that more closely resemble prevalent human inflammatory conditions prompted the present studies to determine whether experimental colitis in rats induced by trinitrobenzene sulfonic acid could provide insight into the mechanisms underlying inflammation and anorexia. A good model for this purpose is defined as one, which leads to a predictable and reproducible course of inflammation, decreased feeding and weight loss. This allows for the full investigation of the relationship between the inflammatory process and any feeding changes observed. The results presented in this thesis demonstrate that the TNB colitis has met the above criteria.

My results indicate that decreased food intake does not reflect a "general malaise". A decrease in meal size with preservation of normal meal frequency is associated with abnormal post-prandial satiety signals and not "general malaise" (143). The initiation of similar number of meals by otherwise anorexic rats clearly show that their is no inability to eat demonstrated by these animals. The decreased intake of liquid and elemental diets, combined with the preservation of pre-treatment water intake, suggest that anorexic animals specifically avoid nutrient intake. This allows for





two interpretations: 1)TNB treated rats become anorexic to deprive their bodies of either general or specific nutritional components in order to deny pathogens micro or macronutrients; or 2)some part of the inflammatory process renders the GI tract incapable of handling nutrient loads and thus anorexia manifests to protect the digestive and absorptive systems compromised by the inflammation. In both of these cases the anorexia could be considered adaptive. Regardless of the adaptive significance of the anorexia, the data presented here demonstrates animals are motivated to eat, begin meals normally and shut-down feeding prematurely, suggesting generation of abnormal gastric or post-gastric satiety signals.

The control of food intake is a heavily researched but poorly understood area of investigation. It is generally accepted that stimulation of chemo- and mechano-receptors in both the stomach (42), duodenum, and ilium (65, 121) results in the liberation of neural and hormonal signals that are integrated in the central nervous system and cause feeding to terminate (109). The results of this thesis suggest involvement of either or both abnormal gastric and post-gastric satiety signals in the manifestation of the anorexia. Sham feeding is a well accepted method for removal of both gastric and post-gastric satiety signals. In my studies, sham feeding TNB and ETOH treated rats showed similar intakes. This indicates that gastric and or post-gastric stimulation is required for the anorexia to present. Previous studies have shown that parasitic infection of the upper GI tract results in disturbed hormone levels and it is possible that CCK or other peptide involved in the control of food intake are altered by the inflammatory process (19). While abnormal post-gastric satiety signal

generation in response to food in the GI tract is a possible explanation for the anorexia, it should be emphasized that there is no inflammation in the proximal small intestine of rats with TNB colitis. For this reason production and release of CCK and other peptides released by the proximal small intestine were not measured. Another possibility is that the anorexia is a direct result of an exaggerated generation of gastric satiety signals. This is supported by the fact that a decreased rate of gastric emptying is observed in anorexic, TNB treated rats.

Although the literature suggests that mechanical distention of the stomach does not contribute to satiety, except at the upper limits of stomach stretch (27), it is possible that increased sensitivity to gastric distention, combined with a decreased rate of gastric emptying, could contribute to the decreased meal size and overall anorexia. While the sham feeding data provides indirect support, decreased gastric emptying is unlikely to be the sole factor mediating the anorexia observed in this model. A causal role of gastric emptying in colitis-induced anorexia could be further assessed with the use of surgical or pharmacological treatments (e.g. pyloroplasty or prokinetic drugs) that normalize the rate of stomach emptying after TNB treatment. I am aware that prokinetic drugs may effect the inflammatory process and the usefulness of pyloroplasty has been questioned as a method to increase the rate of gastric emptying (25).

I speculate that in rats with TNB colitis, anorexia represents a consequence of a specific component of the inflammatory response. In these studies I have used MPO activity as a monitor of the acute inflammatory cell infiltrate in the colon. The data however, indicate a dissociation between MPO activity and decreased food intake.



Examples of this are: 1)the similar MPO levels on day 1 and day 5 post TNB while feeding was only decreased on days 1, 2, and 3; 2)treatment with both indomethacin and rhIL-1ra resulted in attenuation of the anorexia, which had no effect on MPO. Thus, while MPO activity is a convenient and widely used marker of acute inflammation (13, 95, 122), my results show that it is not predictive of the development of anorexia. The attenuation of the anorexia by a receptor antagonist for the pro-inflammatory cytokine IL-1 however, indicates that the anorexia is related to the inflammatory process. I conclude the anorexia results from a component of the inflammatory process not reflected by MPO. That is, the relevant anorexigenic signals are produced either by cells not containing MPO or by MPO containing cells that also produce the anorexigenic signal independently of MPO.

This thesis has also addressed the identity of some putative inflammatory mediators responsible for the anorexia. Treatment with a specific leukotriene synthesis inhibitor, although decreasing tissue $LTB_4$ synthesis in the TNB model, did not alter the profile of anorexia. This leads me to conclude that leukotriene production is not required for the display of anorexia by TNB treated animals. In contrast, treatment with indomethacin significantly attenuated the anorexia seen in TNB treated animals. These data implicate the necessity of prostaglandin production for the full expression of anorexia in this model. As it is generally accepted that prostaglandins are locally acting inflammatory mediators, it is unlikely prostaglandins synthesized in the inflamed colon are the sole mediator of the anorexia in this model.

Exogenous administration of the cytokine IL-1 either peripherally or centrally leads



to a pattern of anorexia similar to that observed with TNB colitis (85). Further, infusion of lipopolysaccharide (a potent stimulus of endogenous IL-1 production) also leads to a pattern of anorexia similar to that seen with TNB and exogenous IL-1 (10). These findings raise the possibility that IL-1 is a mediator of TNB induced anorexia. Additionally, it is known that IL-1 induces prostaglandin synthesis (35) and inhibition of cyclooxygenase blocks exogenous IL-1 induced anorexia (82, 134). Conversely, it has also been shown that PG's are involved in the production and release of IL-1 following stimulation.

The demonstration that peripheral administration of rhIL-1ra partially attenuated TNB anorexia implicates IL-1 as a necessary component of the signals required to produce anorexia. More specifically the attenuation of anorexia by central administration of a dose of rhIL-1ra, that had no effect delivered peripherally, strongly suggests that central IL-1 receptors are involved in the decreased feeding in TNB treated rats. The mechanism by which central IL-1 receptors are stimulated by peripheral inflammation is not understood. The partial attenuation of the anorexia observed with IL-1 antagonism may reflect an insufficient dosage of receptor antagonist. Unfortunately, no further supply of the rhIL-1ra was available. However, the dosages I used have been shown to be effective in other studies to block the effects of high dose exogenous IL-1 (61). The possibility remains, however, that if a higher dose had been used, a complete reversal of the anorexia may have been accomplished. Alternatively, it is possible that other cytokines, such as IL-6 or TNF, which have been shown to have anorexic-like properties, (98, 99, 133), also contribute



to decreased feeding in TNB rats. TNF is especially implicated as it leads to the production of both IL-1 and prostaglandins (4). TNF may also contribute to the weight loss observed in these rats as infusion of TNF has been shown to lead to loss of muscle mass (55). It is also unclear how the occupation of central IL-1 receptors is achieved by inflammation in the periphery. The possibility that IL-1 or other cytokines induce slow wave brain activity and sleep (or other behavioral alterations) should also be investigated as this is a well recognised effect of these mediators (99, 133). It could be the case that the non-reversible portion of the anorexia reflects this behavioral alteration. In summary I have shown in this thesis that TNB induced colitis in the rat is an appropriate model to investigate the process by which inflammation in the periphery can communicate to the brain to alter feeding behaviour. I have argued that the anorexia represents a specific process consequent to the inflammatory process rather than a general malaise. I have also demonstrated that continuity of the GI tract is required for the anorexia to present and that a decreased rate of gastric emptying and or altered sensitivity to distention may contribute to the anorexia. Finally I have demonstrated that production of prostaglandins and occupation of central IL-1 receptors are required for the anorexia to present fully. A final question concerns the relationship between IL-1 released in the periphery as a result of inflammation and IL-1-mediated effects in the brain. It has been reported that radio-labelled IL-1 is transported from the blood to the brain by a shared, saturable mechanism (5). It may be that IL-1 secreted in the periphery is actively transported through the blood-brain barrier into the central nervous system. Alternatively, since IL-1 is not typically

presumed to cross the blood-brain barrier (9, 10, 30). IL-1 may enter the CNS at areas where the blood-brain-barrier is leaky, e.g., the organum vasculosum lateral terminalis or area postrema. Finally, as has been speculated for the induction of fever, peripheral IL-1 may stimulate prostaglandin synthesis in periventricular areas or in the ventricular endothelium, and the increased prostaglandin may consequently stimulate IL-1 production in brain (9). A hypothetical model of how peripherally released Il-1 could communicate with the brain is shown in Figure 21.

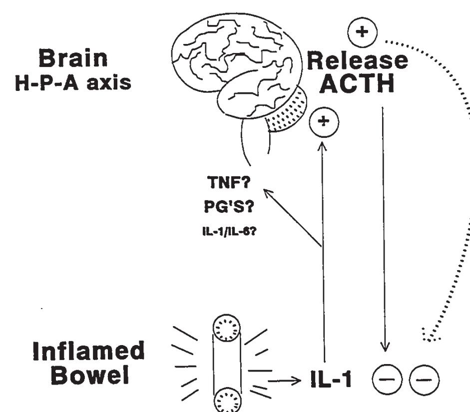


Fig. 21. Hypothetical model of how peripherally released Il-1 could communicate with the brain.



There are several directions for further research suggested by the data in this thesis. The specific brain areas involved in the anorexia should be investigated to see which areas are active (or inactive) when the animals are anorexic. More specifically the presence of IL-1 or other anorexic cytokines in the brain should be examined using immunohistochemical techniques. Following the lead of those researching the physiological effects of exogenous cytokines, the role of corticotrophin releasing factor (CRF) in the brain and activation of the hypothalamic-pituitary axis should be further investigated in this model. Also, as referred to above, the role of other cytokines including IL-6 and TNF should be examined alone and in conjunction with IL-1 receptor blockade to determine whether they are responsible for the IL-1 independent portion of the anorexia.

My results may have implications for the treatment of IBD. The animal literature suggests a possible protective role for inflammation-induced anorexia (88). It has been suggested that animals become anorexic in an effort to decrease the availability of trace metals (132). Studies using administration of total parenteral nutrition in TNB treated rats would be able to address the issue if in fact the anorexia is beneficial in these animals or not, and discern if the anorexia has adaptive significance. An increased mortality or morbidity rate associated with the IV infusion of nutrients would be consistent with the hypothesis that inflammation induced anorexia is protective in the rat. Reversal of malnutrition in IBD patients leads to a decreased need for medication and even surgery in some cases (119). The extent to which anorexia in humans with IBD is adaptive is unclear. Treatment with RhIL-1ra which



successfully attenuated the anorexia in TNB treated rats, is now being investigated in clinical trials in humans with IBD. Unfortunately inhibition of cyclooxygenase cannot be used in the treatment of IBD as use of these drugs have been demonstrated to lead to relapse and/or exacerbations of IBD (40). This data combined with the conflicting results regarding the benefits of anorexia in GI inflammation demonstrates the limitations of using animal models to study human IBD.

This thesis has focused attention on anorexia associated with bowel inflammation. The data presented offers suggestions for future research into the bi-directional link between nutrition and IBD. I believe the research in this thesis, has given insight into a model that is useful not only to investigate the influence of inflammation on feeding behavior but may also be of value as a tool to investigate how peripherally generated signals communicate with the brain to alter behaviour in both health and disease.

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