Role of hypothalamic neuropeptide Y and orexigenic peptides in anorexia associated with experimental colitis in the rat

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ABSTRACT

Neuropeptide Y (NPY) is thought to play a crucial role in the normal hypothalamic response to starvation. After a period of food restriction, increased release of NPY induces hunger and hyperphagia, and helps to restore body weight to its set point. Persistent anorexia in rats with experimental colitis implies failure of this adaptive feeding response. In vivo NPY release and regional hypothalamic NPY concentrations were measured in rats with trinitrobenzene-sulphonic acid (TNBS)-induced colitis, healthy controls and animals pair-fed to match the food intake of the colitic group. Food intake in the colitic group was assessed after administration of NPY and two other potent orexigenic peptides: melanin-concentrating hormone (MCH) and hypocretin (orexin-A). Food intake was decreased by 30–80% below control values for 5 days in the colitic rats. In both the pair-fed and colitic groups, release of NPY in the paraventricular nucleus was significantly increased compared with free-feeding controls. Intraventricular or intrahypothalamic administration of NPY, MCH or hypocretin elicited a feeding response in healthy controls, but not in the colitic group. In summary, animals with TNBS-colitis and anorexia show an appropriate increase in hypothalamic NPYergic activity. However, the failure of NPY and other orexigenic peptides to increase feeding in the colitic group indicates suppression of feeding, either by inhibition of a common downstream hypothalamic neuronal pathway or by induction of one or more potent anorexigenic agents.

INTRODUCTION

Inflammatory bowel diseases, such as ulcerative colitis and Crohn’s disease, and other chronic inflammatory conditions are often associated with marked weight loss. Of adults attending an inflammatory bowel disease clinic, one in five weighed less than 85% of their ideal body weight [1]. Moreover, in children and adolescents with inflammatory bowel disease, weight loss is associated with severe growth impairment and delayed sexual maturation [2–4]. The potential causes of weight loss include decreased energy intake, malabsorption and increased energy expenditure. Of these, prolonged anorexia has been identified as the single most important aetiologic factor [5–7].

In the rat, rectal administration of 2,4,6-trinitrobenzenesulphonic acid (TNBS) induces distal colitis with a macroscopic appearance, histological features and a cytokine profile resembling those of Crohn’s disease [8–10]. Induction of colitis in this model is associated
with anorexia and weight loss, which is thought to be mediated, at least in part, by the interaction of interleukin-1 (IL-1) with one or more central nervous system (CNS) feeding pathways [11–15]. In healthy individuals, weight loss due to underfeeding is a potent stimulus to food intake. Persistent anorexia in underweight animals with TNBS-induced colitis implies a failure of this adaptive feeding response. Numerous factors have been implicated in the CNS control of food intake, including neuropeptide Y (NPY), corticotrophin-releasing factor, monoaminergic transmitters, melanin-concentrating hormone (MCH) and the recently described orexins [16]. Of these, hypothalamic NPY is thought to be a key physiological mediator in the refeeding response after starvation [17,18]. Injection of NPY into the hypothalamus is a potent stimulus to feeding, and prolonged or repeated administration causes sustained hyperphagia and obesity [19,20]. In the hypothalamus, NPY is synthesized predominantly in the arcuate nucleus (ARC) [21]; of the various hypothalamic regions supplied by ARC NPYergic neurons, only the projection to the paraventricular nucleus (PVN) appears to be stimulated in response to a hypocaloric challenge. This results in increased NPY mRNA levels in the ARC, raised NPY levels in the PVN and enhanced NPY release from the PVN [22–25]. In food-deprived rats which have lost weight, the activity of the ARC–PVN projection only returns to normal when overeating has restored body weight to control values [26]. Furthermore, inhibition of NPY synthesis or blockade of the actions of NPY with NPY antisense oligonucleotides or receptor antagonists has been shown to reduce food intake in food-deprived rats [27–29]. NPY is therefore thought to be a key molecule in the hypothalamic response to starvation, and is suggested to drive hunger and hyperphagia after a period of food restriction [17,18]. The failure of rats with TNBS-induced colitis to increase their food intake after weight loss suggests that the NPYergic system may be refractory to stimulation in these animals.

The aim of the present study was to test the hypothesis that anorexia associated with TNBS-induced colitis results from reduced activity of NPY neurons in key hypothalamic regions that have been shown to regulate food intake. In order to achieve these aims, NPY concentrations were measured in microdissected hypothalamic nuclei [30–32], and NPY release was measured in vivo from the PVN [30,32,33]. In addition, food intake was measured in control and colitic rats after intrahypothalamic administration of NPY.

**MATERIALS AND METHODS**

**Animals**

Adult male Wistar rats obtained from Charles River Ltd (Margate, Kent, U.K.) were housed individually at an ambient temperature of 22 ± 1 °C and maintained under a 12 h/12 h light/dark cycle. They were given free access to standard laboratory chow (RMI cubed; Question Nutrition, Canterbury, Kent, U.K.) and tap water. All of the procedures described in this study have been approved under the Animal (Scientific Procedures) Act 1986, Home Office, London, U.K.

**Induction of colitis**

Rats were anaesthetized by intramuscular injection of 0.3 ml of Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml; Janssen Animal Health, High Wycombe, U.K.). In the colitic group, a plastic cannula was inserted 8 cm proximal to the anus for administration of 20 mg of TNBS (Sigma Chemical Co.) in 0.25 ml of 40% (v/v) ethanol [10]. In each experiment, two control groups were used: (1) healthy rats allowed free access to food and water; and (2) a pair-fed group comprising healthy animals whose daily food intake was matched to that of their pair in the colitic group. In the control groups, a plastic catheter was also passed into the colon under anaesthetic and removed after 1 min. Body weight and 24-h food and water intake were measured daily. At the end of the experimental period, animals in the colitic and healthy control groups were killed by cervical dislocation. Via a mid-line laparotomy, a section of the intestine 3 cm proximal to the anus was removed and stored at −20 °C until assayed for myeloperoxidase activity to assess the severity of colitis. The pair-fed rats were killed 24 h after the colitic animals to allow matching of their food intake.

**Hypothalamic microdissection and tissue concentrations of NPY**

A total of 50 male Wistar rats, initial body weight 224 ± 13 g, were divided into three weight-matched groups before the induction of colitis. On day 0, colitis was induced in 20 rats. The colitic animals and 10 healthy animals were allowed free access to food and water. A further 20 animals were pair-fed to match the food intake of the colitic group. Half of the animals in the colitic and pair-fed groups were killed by cervical dislocation 1 day after induction of colitis. The remaining animals in these two groups and the healthy free-feeding controls were killed at day 5.

The brain was rapidly removed and placed on its dorsal surface, and a slice containing the hypothalamus was removed by vertical cuts 1 mm anterior to the optic chiasm and 1 mm posterior to the mamillary bodies. The tissue block was cut into eight slices of 330–500 μm using a vibrating microtome (Campden Instruments Ltd, Loughborough, U.K.). Eight hypothalamic areas (medial preoptic nucleus, lateral preoptic nucleus, PVN, anterior hypothalamic area, ventromedial hypothalamus, dorso-
medial hypothalamicus, lateral hypothalamic area and ARC) were microdissected using a blunt-ended 19-gauge needle and a fine scalpel blade. Tissue from each area was pooled and boiled for 10 min in 400 µl of 0.1 M HCl to extract the peptides. The extracts were frozen at −70 °C until assayed for NPY and protein concentrations.

**Push–pull sampling procedure and release of NPY from the PVN**

Rats were anaesthetized with a mixture of 0.2 ml of Hypnovel (midazolam 5 mg/ml; Roche Products Ltd, Welwyn Garden City, Herts., U.K.) and 0.2 ml of Hypnorm in 0.4 ml of distilled water (intraperitoneal). A 20-gauge thin-walled stainless steel guide cannula (Cooper’s Needleworks Ltd, Birmingham, U.K.) was implanted stereotaxically above the right PVN according to the co-ordinates of Paxinos and Watson [34]: 1.8 mm anterior to the bregma, 0.5 mm lateral from the midline and 7.5 mm below the outer skull surface, with the incisor bar set 3 mm below the horizontal plane. A stainless steel stylet (23 gauge) was inserted into the guide cannula to prevent its blockage, and the animals were then allowed to recover for 5–7 days before perfusions were carried out. However, animals generally recovered fully, with normal food intake, by 2 days after surgery.

A standard concentric perfusion system was used, consisting of a 28-gauge stainless steel inner (push) cannula and a 23-gauge thin-walled outer (pull) cannula. The inner and outer cannulae were connected by PE20 and PE50 tubing (Portex) to 1.0 ml gas-tight Hamilton glass syringes (Sigma) mounted on a modified injection pump (Biotech Instruments Ltd).

For 4 days before push–pull sampling, animals were placed for up to 3 h in the environment used for these experiments. This allowed them to adapt to the experimental conditions and thus help to minimize stress. NPY release was measured in 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 rats before and after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis. Release was also measured in a group of 11 male Wistar rats (body weight 280–320 g) before (control samples) and at four time points after the induction of colitis.

**Food intake after administration of NPY and other orexigenic peptides**

Cannulae were placed into the PVN of six adult male Wistar rats and animals were allowed to adapt to the experimental conditions as described above. The feeding response to NPY administration was determined before and on days 2 and 3 after the induction of colitis. Pig NPY (Bachem Ltd, Saffron Walden, U.K.) was dissolved before use in distilled water and injected into the PVN at a dose (500 ng in 0.25 µl over 1 min) reported to stimulate feeding when administered into the PVN [35]. Animals were placed back in their cages, and 100 g of food was provided in a container of known weight. Water was freely available. Control animals were included in each experiment, and were injected with an equal volume of water. The food remaining was measured at 30 min intervals for 270 min after injection of NPY or water. In subsequent studies, we also tested the feeding response to higher doses of NPY (5 µg and 15 µg) in healthy controls (n = 6) and colitic rats (n = 6) on the second day after induction of colitis. In order to test the specificity of any feeding response to NPY administration in the colitic group, food intake was also measured in a second group of animals after injection of two other orexigenic peptides [36,37]. MCH (15 µg) or orexin-A (hypocretin; 80 µg; Bachem) was administered into the right lateral ventricle before and 2 days after induction of colitis. The time interval between the injection of neuropeptides into controls (i.e. before induction of colitis) and after induction of colitis was at least 5 days. Hypocretin and MCH were injected into the lateral ventricle, because the hypothalamic sites involved in the effects of these peptides on food intake had not been identified when these studies were performed. In contrast, the effects of NPY on feeding are most potent when NPY is injected into the PVN or the adjacent perifornical area [38].

**Measurement of plasma glucose, insulin and corticosterone concentrations**

In a separate study, trunk blood was collected 1 or 5 days after induction of colitis, and from healthy free-feeding controls and pair-fed animals, for glucose and hormone measurement. Blood was collected into EDTA tubes, which were placed on ice until centrifugation at 2000 g at 4 °C for 10 min. Plasma was stored at −20 °C until analysis for glucose and hormone measurements. The glucose and hormone concentrations at day 1 after induction of colitis or pair-feeding have been reported previously [39].
Assays
NPY concentrations in the hypothalamic tissue extracts were measured by RIA using I-{sup}125-I-labelled NPY (Amersham Life Science, Amersham, Bucks., U.K.), rat NPY as standard (Bachem) and rabbit anti-(pig NPY) antiserum at a final dilution of 1:160000 [40,41]. Samples were neutralized with Tris base and diluted in sodium phosphate buffer (pH 7.4) containing 0.5% BSA and 0.01% Triton X-100. The assay sensitivity was 2 fmol/ml, and the intra-assay coefficient of variation was 5%.

Protein concentrations in the hypothalamic extracts were measured after neutralization with 0.1 M NaOH, using the BCA Protein Assay (Pierce, Rockford, IL, U.S.A.). Tissue myeloperoxidase activity was measured using a technique modified [9] from that of Smith and Castro [42].

Plasma glucose concentrations were measured using the GOD-Perid method (Boehringer Mannhein G.m.b.H., Mannheim, Germany). Plasma insulin and corticosterone were measured by RIA (Amersham International plc, Little Chalfont, Bucks., U.K.), both with a within-assay coefficient of variation of 5%.

Statistical analyses
All data are expressed as means ± S.D. unless otherwise stated. Differences in food intake and body weight were compared between the experimental groups by two-way ANOVA. Multiple comparisons were conducted with the Studentized range statistic and evaluated using the Newman–Keuls procedure. Myeloperoxidase concentrations and plasma analyte concentrations were compared using one-way ANOVA coupled to a post hoc Scheffe test. Regional hypothalamic NPY concentrations in the hypothalamic tissue extracts were neutralized with 0.1 M NaOH, were measured by RIA using I-{sup}125-I-labelled NPY as standard (Bachem) and rabbit anti-(pig NPY) antiserum at a final dilution of 1:160000 [40,41]. Samples were neutralized with Tris base and diluted in sodium phosphate buffer (pH 7.4) containing 0.5% BSA and 0.01% Triton X-100. The assay sensitivity was 2 fmol/ml, and the intra-assay coefficient of variation was 5%.

The effects of colonic inflammation on food intake are shown in Figure 1. Intrarectal administration of TNBS significantly suppressed food intake. Group differences in 24-h food intake were significant on each day after induction of colitis (day 1, day 2 and day 3, P < 0.001; day 4, P = 0.002; day 5, P = 0.02). Intake had decreased by 80% on day 1 and, although the degree of anorexia had decreased by day 5, intake was still 33% less than in controls. By definition, food intake in the pair-fed group matched that of the colitic group. In parallel with the suppression of feeding, water intake in the colitic group lost weight. Weight loss in the pair-fed group was similar (P < 0.05) to that in the colitic group (Figure 2). In contrast with the suppression of feeding, water intake in the colitic group was consistently higher than in the healthy controls, but this just failed to reach statistical significance (P = 0.07; Mann–Whitney U test) at any time point. Before induction of colitis, water intake was 41.5 (29.5–70.5) ml/24 h [median (interquartile range)]. At 1 day after induction, water intake was 47.5 (22.5–81) ml/24 h; the maximum water intake in the colitic group was 68 (57–80) ml/24 h, on day 3.
Figure 3  NPY concentrations in eight selected hypothalamic regions in healthy free-feeding controls and in rats 1 day (upper panel) and 5 days (lower panel) after induction of colitis or pair feeding
Values are means ± S.D.; n = 11/group in the upper panel and n = 9/group in the lower panel. Solid bars, healthy free-feeding controls; open bars, rats with TNBS-colitis; grey bars, pair-fed rats. Significance of differences: upper panel, * P = 0.04 compared with controls and † P = 0.009 compared with colitic rats; lower panel, * P = 0.02 compared with controls. MPO, medial preoptic nucleus; LPO, lateral preoptic nucleus; AHA, anterior hypothalamic area; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; LHA, lateral hypothalamic area.

Effects of colitis on regional hypothalamic concentrations of NPY
NPY concentrations in the eight hypothalamic regions at day 1 and day 5 are shown in Figure 3. At day 1, two-way ANOVA revealed a significant group effect (F = 2.58, P = 0.01), a significant difference between regions (F = 93.1, P < 0.0001) and a significant interaction between group and nucleus (F = 5.95, P < 0.007). Regional NPY concentrations were similar among the three groups in all regions, except in the PVN, where NPY concentrations were increased by 35–40 % in the pair-fed group compared with both of the other groups (P = 0.04 compared with controls; P = 0.009 compared with colitic group). PVN NPY concentrations were similar in the control and colitic groups. At day 5, NPY concentrations in the PVN of the pair-fed group were 43 % higher (P < 0.02) than in the controls, but were not significantly different from those in the colitic group.

Effects of colitis on release of NPY from the PVN
Similar to the results in the preceding experiment, animals with colitis showed a marked suppression of feeding associated with weight loss and a significant increase in intestinal myeloperoxidase concentrations. Figure 4 shows the mean level of PVN NPY release over the entire 150 min sampling period from controls, and for 6 days after induction of colitis or pair-feeding. As expected, food restriction in healthy animals (i.e. pair-feeding) resulted in increased release of NPY from the PVN, with a 2–3-fold increase over control values. Furthermore, there was no significant difference between NPY release in the pair-fed group (with an increased drive to eat) and the colitic group, who had marked suppression of feeding. In addition, the pattern of release in pair-fed and colitic rats was similar, but differed from that in healthy free-feeding controls. NPY release in the PVN of free-feeding control rats was at a stable low rate throughout the sampling period (Figure 5). However, in both colitic and pair-fed rats, NPY release occurred in pulses.

Effects of orexigenic peptides on feeding behaviour in colitis
Administration of NPY, MCH or hypocretin increased food intake in control animals (Figure 6). Administration of NPY (500 ng) led to an approx. 2-fold increase in food intake at all time points compared with placebo-treated
controls \((P < 0.001)\). Intracerebroventricular administration of MCH also increased food intake in control animals at all time points \((P < 0.01)\), but hypocretin had only a weak effect on food intake, with a significant \((P = 0.006)\) increase only after 270 min compared with placebo-treated controls. However, the purpose of this study was not to compare the potency of these peptides in healthy animals, and therefore no direct comparison can be made. In contrast, administration of NPY, MCH or hypocretin did not increase feeding in the colitic group. We subsequently tested the effects of higher doses of NPY \((5 \text{ and } 15 \mu g)\) on feeding in rats with TNBS-colitis. As with the lower dose of NPY, a robust feeding response was seen in healthy controls, but not in colitic animals. At a dose of \(15 \mu g\), healthy controls ate \(7.5 \pm 2.3\) g of food in 2 h, compared with only \(1.5 \pm 0.7\) g in the colitic group.

### Plasma glucose, insulin and corticosterone concentrations

At 1 day after induction of colitis, plasma glucose concentrations were reduced by \(30\% \ (P = 0.006)\) in the colitic group and by \(37\% \ (P = 0.001)\) in the pair-fed group, when compared with controls (Table 1). There was no difference between the pair-fed and colitic groups. Plasma insulin concentrations were reduced by \(76\%\) in the pair-fed group \((P = 0.001)\) compared with both the colitic and control groups. Plasma corticosterone concentrations were over 2-fold higher in the colitic group compared with controls \((P < 0.006)\), but there was no significant difference between the control and pair-fed groups \((P = 0.06)\).

At 5 days after the induction of colitis, plasma insulin concentrations were lower than control values in both the pair-fed and colitic groups. Glucose concentrations were lower than controls only in the pair-fed group.

### DISCUSSION

We have confirmed that the intrarectal administration of TNBS to rats causes marked distal colitis and suppression of feeding [9]. Intravenous administration of TNBS in ethanol did not reduce food intake (A. B. Ballinger and O. Azooz, unpublished work), suggesting that hypophagia in TNBS-colitis is due to the inflammatory response induced and not to a direct systemic effect of TNBS. Weight loss was similar in the colitic and pair-fed groups, suggesting that weight loss results primarily from anorexia and not from an associated increase in energy.

<table>
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<tr>
<th>Table 1</th>
<th>Plasma concentrations of glucose, insulin and corticosterone in healthy control animals and in rats at 1 and 5 days after induction of colitis or pair feeding</th>
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<td></td>
<td>Controls</td>
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<tr>
<td>Glucose (mmol/l)</td>
<td>8.2 ± 0.54</td>
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<tr>
<td>Insulin (ng/ml)</td>
<td>1.34 ± 0.12</td>
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<tr>
<td>Corticosterone (ng/ml)</td>
<td>153 ± 41</td>
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suggests that anorexia may be a result of an alteration in appetite, however, this is not inducible by NPY and other orexigenic peptides. The observation that feeding behaviour in colitic rats is increased synthesis or transport is presumably a transient effect, because overall our results indicate that systemic inflammation does not inhibit the increase in PVN NPY release.

Within the rat hypothalamus, NPY is synthesized mainly in neurons of the ARC. Axons from these cells project through the lateral hypothalamus to end in the PVN and the dorsomedial hypothalamus. At least three observations suggest that the PVN is crucial for stimulation of feeding by NPY. First, NPY concentrations increase in the PVN in response to food deprivation; secondly, administration of NPY into various hypothalamic regions increases food intake, but the feeding response is particularly robust after injection into the PVN and the adjacent peri-fornical area; and finally, intracerebroventricular injection of NPY results in stimulation of feeding and intense c-fos expression in the PVN.

We have tested the hypothesis that anorexia associated with TNBS-induced colitis results from reduced activity of PVN neurons and that, based on this premise, food intake would increase after administration of NPY into the PVN. As expected, in healthy but food-restricted animals (i.e. pair-fed to match the hypophagia of the colitic rats), NPY concentrations and release in the PVN were consistently higher than in free-feeding controls. However, in contrast with our predictions, NPY release was also increased in the animals with colitis, all of whom had severe anorexia.

The concentration of a peptide within a presynaptic nerve terminal reflects the dynamics of synthesis, transport to the nucleus, storage, release and degradation. Both hypothalamic ARC and brainstem neurons provide NPY afferents to the PVN. However, the normal feeding response induced by food deprivation depends on the supply of NPY from the ARC. In the pair-fed group, the concentration of NPY and its release were increased in the PVN at both time points, presumably as a result of increased synthesis in the ARC and increased transport of NPY to the PVN. In the colitic group, NPY concentrations in the PVN, but not release, were lower than in pair-fed group on the first day after induction of colitis. This discrepancy between content and release indicates that turnover may be increased compared with controls, but that the level of synthesis is less than in pair-fed animals, possibly as a result of the effect of inflammatory cytokines. However, any abnormality of peptide synthesis or transport is presumably a transient effect, because overall our results indicate that systemic inflammation does not inhibit the increase in PVN NPY release that normally occurs in energy-deficient states.

An appropriate increase in NPY release, together with the observation that feeding behaviour in colitic rats cannot be induced by NPY and other orexigenic peptides, suggests that anorexia may be a result of an alteration in a final common pathway utilized by all of these orexigenic signals. Alternatively, feeding may be inhibited by the increased activity of a highly potent anorexic signal, either one that is normally present (for instance 5-hydroxytryptamine (serotonin) or corticotrophin-releasing factor) or one that is only induced in the presence of inflammation. We have reported previously that medial hypothalamic 5-hydroxytryptamine contributes to anorexia in the TNBS-colitis model, but is not the only mediator. The present study is the first to examine NPY neuronal activity and response to NPY administration in a model of inflammation where anorexia is clearly cytokine-mediated. IL-1 is one of the pro-inflammatory cytokines that is implicated in the anorexia and weight loss associated with TNBS-colitis. First, the pattern of anorexia after TNBS treatment is similar to that seen on chronic administration of IL-1. Secondly, intestinal IL-1 concentrations peak 24 h after the induction of experimental colitis, when the suppression of feeding is maximal, and patients with inflammatory bowel disease show elevated IL-1 concentrations in colonic biopsies and serum. Finally, central administration of an IL-1 receptor antagonist significantly attenuates anorexia and weight loss in TNBS-colitis, suggesting that CNS IL-1 receptors might mediate anorexia in this model. However, the results of previous studies that have examined the NPYergic system after exogenous administration of IL-1 cannot necessarily be extrapolated to disease models where hypothalamic concentrations of IL-1 may differ by an order of magnitude and where other cytokines can exhibit additive or synergistic activities.

Nevertheless, in agreement with our data, intracerebroventricular administration of IL-1β attenuates or blocks the NPY-induced increase in feeding. Tumour necrosis factor-α (TNF-α), another pro-inflammatory cytokine, is also implicated in the pathogenesis of anorexia and weight loss that occurs in some other chronic diseases, although its role has not been examined in the colitis model. An interaction between NPY and TNF-α has not been explored in detail, although in vitro studies have shown that TNF-α does not inhibit release of NPY.

In the pair-fed group we found reduced plasma insulin concentrations and increased corticosterone concentrations. These hormonal mediators of the peripheral response to energy deficit also provide afferent information to the hypothalamus, where a co-ordinated response leads to an increased drive to feeding. At 1 day after induction of colitis, the plasma concentrations of insulin remained similar to control values. The insulin concentrations in the colitic group were therefore inappropriately raised in the presence of restricted feeding and weight loss. The raised insulin concentrations in the colitic group may be one of the factors that prevent the normal feeding response to a hypocaloric challenge.
In addition, plasma concentrations of leptin, an adipocyte-derived hormone which regulates food intake and energy balance, are transiently increased in TNBS-colitis, and may also contribute to the development of anorexia [52]. In contrast with our findings in animals with intestinal inflammation, reduced NPYergic activity has been reported in tumour-bearing rats [18,53,54]. However, loss of appetite in this model of cancer anorexia is more sustained than in TNBS-colitis, and the role of IL-1, if any, has not been defined. Thus the aetiology of anorexia associated with malignancy may differ from that associated with inflammatory disease. Tumour-bearing rats are, however, refractory to NPY-induced feeding, but their response to other orexigenic peptides has not been tested [35].

In summary, we have shown that TNBS-colitis induces weight loss which is due entirely to anorexia and reduced food intake. Release of PVN NPY is similar to that in food-restricted healthy animals, but NPY-induced feeding, even at high doses, is refractory in colitic animals. These results suggest that anorexia associated with colitis is mediated by an abnormality of NPY receptors or their associated intracellular signalling cascade, or by dysregulation of one or more other factors that control feeding. In the present study we have examined the interaction between intestinal inflammation and CNS feeding pathways, and therefore our experiments had to be performed in intact animals. However, several studies suggest that the TNBS-colitis model, in the early stages of evolution, is similar to human Crohn’s disease, particularly with respect to T cell activation and cytokine profile [8–10,12–14,55]. Thus we believe that our results may be extrapolated to humans in a meaningful way.

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