Antinerve Growth Factor Treatment Prevents Intestinal Dysmotility in *Trichinella spiralis*-Infected Rats

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**ABSTRACT**

Nerve growth factor (NGF) could be involved in the development of hyperalgesia as well as in nervous remodeling consequence of inflammation. Both dysmotility and increase of visceral sensitivity have been described in functional gastrointestinal disorders such as irritable bowel syndrome. *Trichinella spiralis*-infected rats show an exacerbated response to cholecystokinin (CCK), both associated with a reversible inflammatory process and the hypertrophy of the muscle layers. In this study we determined the intestinal expression of NGF mRNA by polymerase chain reaction and NGF by enzyme-linked immunosorbent assay. We implanted serosal stimulation. The experimental protocol included the evaluation of intestinal spontaneous motor activity (SMA), the response to CCK, and the ascending contraction induced by electrical mucosal stimulation. This protocol was performed in healthy and infected nontreated rats, in healthy rats with an NGF antibody treatment (1.6 mg/rat i.p.), and in infected rats with the same treatment applied at 0 or 3 days postinfection. NGF and NGF mRNA levels in the bowel were increased during inflammation. Although anti-NGF treatments did not prevent or reverse inflammatory response, the treatment was effective in preventing the motor alterations induced by the *T. spiralis* infection, i.e., inhibited increased SMA, reversed altered response to CCK, and reversed in part exacerbated response to electrical stimulation.

Motor alterations associated with clinical symptoms such as diarrhea, constipation, and abdominal pain have been described in patients affected by irritable bowel syndrome (IBS) (Harvey and Read, 1973; Azpiroz, 1999). Although IBS pathogenesis is still undetermined, increased sensitivity to gastrointestinal reflexes resulting from nerve remodeling after a remote infection has been suggested as a possible cause of these symptoms (Azpiroz, 1999; Camilleri, 2001). Both intestinal hypermotility and nerve remodeling have been described in several animal models using experimental parasite infection (Castro et al., 1976; Palmer et al., 1984; Stead, 1992). In fact, the adaptive response to intestinal parasites has been suggested as a paradigmatic defense response of the intestine against external pathogens. For this reason, experimental parasite infection has been widely used as model to understand pathogenesis of intestinal functional disorders (Blennerhassett et al., 1992; Stead, 1992; Hogaboam et al., 1996; Torrents and Vergara, 2000).

Recently, we demonstrated that *Trichinella spiralis*-infected rats show an increased spontaneous motor activity (SMA), increased ascending contraction of the peristaltic reflex, and an abnormal response to cholecystokinin (CCK) (Torrents and Vergara, 2000). The control mechanisms involved in these responses indicated that both intrinsic and extrinsic nervous systems of the bowel can be altered during intestinal response to the parasite and become the cause of the motor disturbances.

Nerve growth factor (NGF) is a protein of the family of neurotrophins involved in the functionality of sensory nerves (Urschel et al., 1991), and it has been implicated in the development of hyperalgesia during inflammation (Lewin et al., 1994; Woolf et al., 1994; McMahon, 1996; Theodosiou et al., 1999). The expression of both NGF and its receptors by immune cells such as mast cells, lymphocytes, and basophils has been described previously (Leon et al., 1994; Otten et al., 1996; Palmer et al., 1984; Castro et al., 1976; Stead, 1992; Hogaboam et al., 1996; Torrents and Vergara, 2000). The control mechanisms involved in these responses indicated that both intrinsic and extrinsic nervous systems of the bowel can be altered during intestinal response to the parasite and become the cause of the motor disturbances.

**ABBREVIATIONS:** IBS, irritable bowel syndrome; SMA, spontaneous motor activity; CCK, cholecystokinin; NGF, nerve growth factor; ELISA, enzyme-linked immunosorbent assay; PI, postinfection; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; cNT, nontreated healthy rats; cNGF, healthy rats treated with polyclonal neutralizing NGF; INT, nontreated infected rats; IgG, infected rats treated with an unspecific IgG; NGF0, infected rats treated with anti-NGF 1 h before infection; NGF3, infected rats treated with anti-NGF on day 3 postinfection; EMS, electrical mucosal stimulation; L-NNA, N^\text{\textregistered}-nitro-L-arginine; c/h, contractions per hour; NO, nitric oxide; Ach, acetylcholine.
1994; Levi-Montalcini et al., 1996; Sawada et al., 2000). Moreover, a correlation between proliferation of vagal pathways and hyperplasia of mast cells has been demonstrated in experimental parasite infection (Stead, 1992).

Previous studies showed the presence of NGF in the intestine of adult rats (Weskamp and Otten, 1987) and its production in vitro by intestinal epithelial cells (Varilek et al., 1995). Moreover, a recent study in a colitis model in rats showed a protective role of NGF (Reinhagen et al., 2000). However, the involvement of NGF in the nerve remodeling associated to intestinal hypermotility has not been studied yet.

The hypothesis of our study was that NGF might be involved in the genesis of hypermotility consequence of parasite infection and therefore in the exacerbation of intestinal reflexes observed in functional gastrointestinal disorders. Thus, the objectives of this study were to 1) determine the presence of NGF in the rat intestine and the possible overexpression of this neurotrophin during intestinal inflammation; 2) elucidate the possible role of NGF in the in vivo motor alterations described in T. spiralis-infected rats by means of a treatment with anti-NGF antibodies; 3) evaluate either the preventing or therapeutic value of this immunoneutralization using two different schedules for treatment, before and after inflammation was developed; and 4) evaluate by histopathology the severity of the inflammation.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River, Lyon, France), 8 to 10 weeks old and weighing 300 to 350 g, were used in this study. They were kept under conventional conditions in a room with controlled temperature and photoperiod (12:12 h). Animals were specific pathogen free when purchased, and during the experimental period they were checked for absence of intestinal parasites. Animal weight as well as food and water consumption were monitored daily.

T. spiralis Infection. Rats were infected by administering 1.0 ml of 0.9% saline solution containing 7500 T. spiralis larvae by gavage. The larvae were obtained from female CD1 mice infected 30 to 90 days before by a modification of the method described by Castro and Fairbairn (1969).

NGF mRNA Determination and NGF ELISA Assay. For NGF mRNA and protein determination, tissue samples from the jejunum of healthy rats and of 3 day postinfection (PI)-infected rats were taken immediately after killing the animals. In accordance with approval euthanasia procedures, animals were first stunned and then killed by decapitation. A 10-cm segment of the mid-jejunum was cut and divided into four equal parts. Each sample was then weighted and stored immediately at −70°C.

Reverse transcriptase-polymerase chain reaction was used to analyze tissue NGF mRNA levels. Total RNA was extracted from the samples using TriReagent (Biotecx, Houston, TX) according to the manufacturer’s instructions. Before the single-strand synthesis we treated the total RNA with DNA-free (Ambion, Madison, WI) 30 min at 37°C to remove any genomic DNA contamination. cDNA was synthesized from 5 μg of total RNA in a reaction mixture of 50 μl containing 0.5 μg of oligo15dT primer (Promega, Austin, TX), 0.4 mM dNTP (Ecogen, Barcelona, Spain), 10 μM dithiothreitol, and 10 U of Moloney murine leukemia virus (both of Epicenter, Madison, WI).

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The resultant cDNA was amplified in a total volume of 50 μl with 1 U of TqgDNA and 0.2 mM dNTP mixture, and 0.4 μM of NGF primers (Little et al., 1994) generating a fragment of 288 pb. As a control of the efficiency of cDNA synthesis we amplified GAPDH (upper primer 5′-gagccccctgcctgtagtcg-3′ and lower primer 5′-atgagc-

ccctacagcatgtc-3′), generating a fragment of 140 pb to test the efficiency of cDNA synthesis. The polymerase chain reaction amplification protocol was as follows: 35 cycles at 95°C for 1 min, 55 or 50°C (NGF or GAPDH, respectively) for 1 min, and 72°C for 1 min; the final extension was for 5 min at 72°C. Amplified products were electrophoresed on 2% agarose gel, stained with ethidium bromide, and photographed under UV light. To have a semiquantitative measure of mRNA NGF we calculated a ratio between band intensity of NGF and GAPDH for each sample.

For NGF protein extraction the frozen samples were ground up into a powder with a pestle and mortar, and then resuspended in extraction buffer (1.5 g of intestine/ml of buffer) containing the protease inhibitors phenylmethylsulfonylic acid (0.1 mM), benzethonium chloride (0.1 mM), aprotinin (7 μM/ml), and EDTA (4 mM) (all purchased from Sigma-Aldrich, St. Louis, MO). The NGF ELISA assay (Roche Applied Science, Manheim, Germany) was used according to the manufacturer’s instructions to determine the amount of NGF in rat tissues. Briefly, anti (2.5 S, 7S)-NGF antibody (clone 27/21) was used as a capture antibody at a concentration of 0.67 μg/ml. Unspecific binding was prevented with 0.5% bovine serum albumin in binding buffer at 37°C for 30 min and washed eight times before adding samples and standards. The plates were incubated overnight at 4°C and then washed extensively. β-Galactosidase-linked anti-β (2.5 S, 7S)-NGF antibody (clone 27/21) was applied (4 U/ml) and incubated for 4 h at 37°C. The color intensity was determined photometrically (570 nm) after 30 min of incubation at 37°C with the substrate (chlorophenol red-β-d-galactopyranoside). For each plate a mouse NGF-β standard curve was performed in parallel.

Animal Groups and NGF Treatments. Six groups of rats were studied: 1) nontreated healthy rats (cNT) (n = 6); 2) healthy rats treated with polyclonal neutralizing NGF (anti-NGF) antibody (Sigma-Aldrich) (cNGF) (n = 4); 3) nontreated infected rats (iNT) (n = 6); 4) infected rats treated with an unspecified IgG (Sigma-Aldrich) (IgG) (n = 4); 5) infected rats treated with anti-NGF 1 h before infection (NGF0) (n = 4); and 6) infected rats treated with anti-NGF on day 3 PI (NGF3) (n = 6).

Both anti-NGF and IgG treatments consisted of a single intraperitoneal dose of 1.6 mg of the correspondent antibody in 1 ml of sterile 0.9% saline solution. These doses were calculated from data from a previous study using the same antibody (Reinhagen et al., 2000). Because both animal experimental data (Watkins et al., 1997; Murphy et al., 1999) and protocols with antibodies followed in humans (Targan, 2000) prove that IgG remains in high concentrations in blood for prolonged periods, we considered a single injection sufficient to maintain IgG concentration for the duration of the experiment.

Animal Preparation for Motility Studies. After a fasting period of 12 to 16 h, animals were prepared for the experimental procedures as described previously (Torrents and Vergara, 2000). Briefly, anesthesia was induced by inhalation of halothane to allow cannulation of the right jugular vein. Level III of anesthesia was maintained for the rest of the experimental protocol with thiopental sodium bolus infusion in the jugular vein as required. Body temperature was maintained at 37°C by placing the animal on a heating pad. The abdomen was opened and three strain-gauges 3 × 5 mm (Hugo Sachs Elektronik, March-Hustetten, Germany) were placed to record circular muscle activity and sutured to the intestinal wall of the duodenum, proximal jejunum, and ileum, respectively. Strain-gauges were connected to high-gain amplifiers (MTS-P; Lectromed Ltd, Letchworth, Herts, UK), and amplified signals were sent to a recording unit (PowerLab/800; ADInstruments Pty Ltd., Castle Hill, Australia) connected to a PC running PowerLab software. Finally, two electrode holders each with two platinum electrodes (WPI, Sarasota, FL) were inserted into the intestinal lumen at 1 cm distally to the strain-gauge of the duodenum and the ileum, respectively, to induce ascending excitation of the peristaltic reflex by mucosal electrical stimulation (EMS) as described previously (Giralta and Ver-
gara, 2000). The Ethical Committee of the Universitat Autònoma de Barcelona approved all experimental procedures.

**Evaluation of Motor Parameters.** After an equilibration period of 20 min, spontaneous motor activity (SMA) was evaluated for 1 h. Then, CCK-8 (Peptide Institute, Inc., Osaka, Japan) (3 × 10^{-9} mol/kg/10 min) was i.a. in a 2 ml of saline solution with 5% Tween 80 and 5% dimethyl sulfoxide) and saline solution alone (7.2 mg/kg in 2 ml of saline solution with 5% Tween 80 and 5% dimethyl sulfoxide) and saline solution alone (0.09 ± 0.05 pg of NGF/ml; n = 6). Intestinal samples from infected animals also showed increased levels of NGF mRNA expression (1.088 ± 0.06; n = 6) compared with healthy rats (0.71 ± 0.13; n = 3).

**Intestine SMA.** Spontaneous activity was different in healthy and infected rats. In infected rats, a clustered pattern substituted the single contractions observed in the intestine of healthy rats. In consequence, the analysis of contraction frequency showed a significant difference (12.9 ± 3.4 c/h in cNT versus 29.3 ± 4.6 c/h in iNT). This abnormal motility was reversed by early treatment with anti-NGF (9 ± 3.5 c/h in NGF0) but not by the late treatment with the anti-NGF (39.4 ± 7.6 c/h in NGF3) or with specific IgG (30 ± 7.1 c/h in IgG). Treatment of healthy rats with NGF antibody did not cause any alteration on the SMA (14.3 ± 2.2 c/h in cNGF). Statistical differences are shown in Table 1.

**NGF ELISA Assay and NGF mRNA Determination.** Both NGF protein and mRNA levels were higher in the intestinal tissue from infected rats than in healthy control rats (Fig. 1, A and B, respectively). Samples from infected animals showed increased levels of NGF (0.85 ± 0.26 pg of NGF/ml; n = 6) compared with samples from healthy rats (0.09 ± 0.05 pg of NGF/ml; n = 6). Intestinal samples from infected animals also showed increased levels of NGF mRNA expression (1.088 ± 0.06; n = 6) compared with healthy rats (0.71 ± 0.13; n = 3).

**Results**

**TABLE 1**

<table>
<thead>
<tr>
<th>SMA of duodenum and motor response of duodenum (CCK-D) and jejunum (CCK-J) to CCK administration</th>
<th>cNT</th>
<th>cNGF</th>
<th>IgG</th>
<th>NGF0</th>
<th>NGF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>12.9 ± 3.4</td>
<td>14.3 ± 2.2</td>
<td>29.3 ± 4.6*</td>
<td>30 ± 7.1*</td>
<td>9 ± 3.5</td>
</tr>
<tr>
<td>CCK-D</td>
<td>340 ± 45</td>
<td>430 ± 133</td>
<td>709 ± 87*</td>
<td>728 ± 301*</td>
<td>210 ± 124</td>
</tr>
<tr>
<td>CCK-J</td>
<td>4.6 ± 2.8</td>
<td>4.3 ± 3</td>
<td>128 ± 53*</td>
<td>140 ± 37*</td>
<td>18.2 ± 13</td>
</tr>
</tbody>
</table>

* Significant differences compared with cNT group values (p ≤ 0.05).
and an example of these results is shown in Fig. 2. After L-NNA infusion during the experimental protocol an increase of spontaneous motor activity was observed in all the groups without any significant difference between them (data not shown).

**Response to CCK and Capsaicin Studies.** CCK induced an abnormal response in the intestine of nontreated infected animals and in those treated with the unspecific IgG. This response consisted of an increased excitatory response in the duodenum concomitant with an excitatory response of the jejunum. In contrast, both groups of animals treated with the NGF antibody showed a response that was similar to noninfected animals. This response is characterized by the excitation of the duodenum while the jejunum remains quiescent (Fig. 3; Table 1).

Intraluminal instillation of capsaicin abolished the CCK excitatory response on duodenum both in healthy and infected rats. Moreover, capsaicin also completely blocked the excitatory jejunal response to CCK in infected rats. Instillation of the solvents without capsaicin did not modify CCK response (sham) (Fig. 4).

**Response to EMS.** Duodenal response to EMS (Fig. 5) increased during intestinal inflammation both at low frequency, 2 Hz, and at high frequency, 6 Hz. Only the early treatment with anti-NGF (NGF0) reverted the exacerbated response to 2 Hz.

In cNT rats, L-NNA infusion enhanced duodenal response to EMS both at 2 and at 6 Hz. This enhancement of the response was absent in iNT rats at 2 Hz but not at 6 Hz. This effect was reversed at 2 Hz by early treatment with anti-NGF (NGF0), but not by late treatment with anti-NGF (NGF3). Atropine infusion significantly decreased the duodenal response to EMS in all groups and both at 2 and 6 Hz. However, atropine-resistant response in infected animals was of greater amplitude. This effect was reversed by both anti-NGF treatments.

Ileal response to EMS (Fig. 6) also increased in iNT rats at 6 Hz but not at 2 Hz. In addition, none of the treatments modified the characteristic response of each state. At 2 Hz, L-NNA infusion produced a significant increase of the response to EMS in iNT rats but not in cNT rats. Both treatments with NGF antibody reversed this alteration. Thus, L-NNA in NGF0 and NGF3 rats did not significantly enhance the ileal response to EMS at 2 Hz. The same effect was observed in the NGF0 group at 2 Hz.

In the ileum, atropine resistant response increased in infected animals. Both NGF antibody treatments were effective in reversing this effect. Anti-NGF effect was more remarkable at 2 Hz.

**Histological Study.** Intensity of the inflammatory lesions and thickness of circular muscle layer observed in the different groups is summarized in Table 2. In cNT and in cNGF groups no lesions were observed in the jejunal mucosa and submucosa. In contrast, in iNT, and in animals infected with NGF or IgG unspecific, a severe mixed, but mainly mononuclear inflammatory cell infiltrate, was present in the lamina propria, submucosa, and to a lower...
extent in the smooth muscular layers of the jejunum. Moreover, intestinal inflammation was accompanied by a smooth muscle hypertrophy of the intestinal muscle layers. This hypertrophy was not reversed by any of the treatments used in this study.

Discussion

This study demonstrates that NGF is overexpressed in the small intestine as a consequence of *T. spiralis* infection and also that this neurotrophin plays an important role in the development of motor alterations. Furthermore, because treatment with an antibody against NGF either prevented or attenuated most of the abnormal motor changes, this treatment may be a useful therapy for motor disorders caused by hypermotility.

As shown by ELISA, the infected intestine had a 4-fold increase of NGF protein versus the control tissue. This was due to a higher NGF production in the gut as revealed by the increased level of mRNA. This higher amount of NGF in inflamed tissue has also been described in other inflammatory models such as rheumatoid arthritis (Aloe et al., 1992), pancreatitis (Toma et al., 2000), and experimental colitis (Reinshagen et al., 2000); and has been associated with the increased nervous sensitivity during inflammation (Woolf et al., 1994).

In our case NGF overexpression was already significant at 3 days PI. However, preliminary data from intestine samples at 12 and 18 h already showed a slight increase on NGF mRNA (data not shown). These results indicate that NGF overexpression is initiated soon after inflammation and probably causes nerve remodeling necessary to induce hypermotility that is already very significant in this model around 6 days PI (Torrents and Vergara, 2000). Our treatment schedule was planned to prevent NGF action (NGF0) or to block NGF when it was already overexpressed (NGF3).

The intestinal motor hyperactivity induced by *T. spiralis* is a well known model of intestinal hypersensitivity. Inflammation, as a consequence of parasites invading the mucosa, starts a few hours after infection, and it is restricted to duodenum and jejunum mucosa (Dick and Silver, 1980). However, hypermotility, characterized by clustered contractions, appears around day 6 PI and reaches its peak around day 11 PI, concomitant with parasite expulsion. In addition, both hyperplasia and hypertrophy of the muscular layers of the whole small intestine, including the ileum, are significant around day 6 PI but last more than 72 days, far longer than the parasite expulsion and inflammation resolution (Torrents and Vergara, 2000). We chose to perform the experiments at 10 to 12 days PI when hypermotility was fully developed.

Anti-NGF preventive treatment (NGF0) completely blocked the development of spontaneous hypermotility, clearly indicating that NGF has a role in the development of abnormal motility. The fact that the NGF3 group presented a hypermotility similar to infected nontreated animals indicates that treatment was unable to revert completely motor changes once inflammatory mechanisms were activated. However, spontaneous motor activity is a complex event where both...
Histological study: severity of the inflammatory mixed infiltrate in the mucosa and submucosa of jejunum and its circular muscle thickness

<table>
<thead>
<tr>
<th>Lesions intensity</th>
<th>cNT</th>
<th>cNGF</th>
<th>iNT</th>
<th>iIgG</th>
<th>NGF0</th>
<th>NGF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle thickness</td>
<td>46.2 ± 2.4</td>
<td>41.7 ± 1.6</td>
<td>112.2 ± 9.1**</td>
<td>123.6 ± 6.7**</td>
<td>120.3 ± 15.3**</td>
<td>105.4 ± 16.9**</td>
</tr>
</tbody>
</table>

- , absence; +, slight; ++, moderate; ++++, intense (lesion intensity).

**P < 0.01 compared with the cNT value.

intrinsinc and extrinsic pathways are involved. Thus, it is necessary to study other parameters that allow identification of the diverse mechanisms involved. For this reason, we also studied the response to CCK and to electrical stimulation.

We have already described in detail the mechanisms of the response to CCK in the experimental model used in this study: The excitatory response is mediated by mucosal afferent stimulation, whereas the inhibitory response observed in the jejunum is due to stimulation of NO release at the neuromuscular level (Giralt and Vergara, 1999). During inflammation, CCK excitatory response in the duodenum increases and the jejunum strongly contacts instead of being inhibited (Torrets and Vergara, 2000). In the present study we demonstrate that abnormal response to CCK is also mediated by capsaicin-sensitive afferent fibers, indicating that the abnormal response to CCK is due to an exacerbated response of afferent innervation.

Both anti-NGF treatments, either at time 0 (NGF0) or once inflammation was established (NGF3), were effective in preventing CCK abnormal response. This indicates that exacerbation of afferent response is dependent of the overexpression of NGF. Furthermore, NGF antibodies could be a possible treatment for those intestinal syndromes where there is abnormal hypersensitivity, such as IBS where abnormal responses to CCK have also been described previously (Harvey and Read, 1973). However, from our study we cannot know whether anti-NGF treatment prevented either the proliferation of afferent fibers described during intestinal inflammation (Sharkey, 1992; Shea-Donahue et al., 1997) or the hyperplasia of other cell types, such as mast cells, which also increase in numbers in this experimental model and that have been suggested as a cause of afferent proliferation (Stead, 1992).

Intraluminal electrical stimulation elicits the ascending contraction of the peristaltic reflex. In contrast to CCK response and to spontaneous motility, this response can be blocked by intraluminal application of lidocaine but not by capsaicin (Giralt and Vergara, 2000). In consequence, electrical stimulation acts on either capsaicin-insensitive afferents or, more likely, on a wide number of neurons from the submucous plexus.

Ascending contraction is enhanced in infected animals. The response to electrical stimulation has been extensively studied in several models of intestinal disease both in vivo and in vitro conditions, and three hypothesis have been raised to explain the hyper-response observed: 1) an impairment of the inhibitory innervation (Hogaboam et al., 1996); 2) the alteration of the acetylcholine (Ach)/substance P response (Collins et al., 1989); and 3) the hypertrophy of muscle layers (Blennerhasset et al., 1992).

In relation to whether there is an impairment of NO innervation, our results are not conclusive. Infusion of 1-NNA gave a similar increase of spontaneous motility in all experimental groups. However, at 2 Hz, a frequency of stimulus that recruits inhibitory innervation (Daniel and Kostolanska, 1989), 1-NNA did not increase ascending excitation, suggesting an impairment of NO. Anti-NGF preventive treatment (NGF0) brought back 1-NNA effect, indicating a restoration of NO response. In a previous study, also using the same experimental model (Torrets and Vergara, 2000), we reported that there was not a clear alteration of NO innervation that could explain the hyper-response observed. However, from the present study we can conclude that if there is a certain functional impairment of NO innervation, it ameliorates after anti-NGF treatment.

In addition, the increased ascending contraction could also be a consequence of the remodeling of excitatory innervation. The excitatory intrinsic network of the intestine has been extensively studied. Both Ach and substance P are colocalized in the same intrinsic motor neurons (Furness et al., 1992), and changes in the Ach versus the substance P component of the ascending contraction have been suggested after T. spiralis infection (Torrets and Vergara, 2000). Moreover, an increase of substance P concomitant with a decrease of acetylcholine has been reported (Collins et al., 1989; Swain et al., 1992). In the present study, both anti-NGF treatments, but mainly the preventive one, reverted the
increased response after atropine, allowing us to postulate that NGF is also responsible for the remodeling of Ach/substance P neurons.

In contrast, none of the treatments prevented hypertrophy of the muscle. Hypertrophy could have contributed to the fact that none of the treatments was completely effective in reverting ascending contraction.

Similarly, none of the anti-NGF treatments were effective in preventing inflammation. This finding is interesting because it causes that nerve remodeling is a specific action of NGF and not a secondary phenomenon of inflammation. It could also explain why in some hyper-responsive syndromes of the intestine score of inflammation is not well correlated with to the severity of the symptoms. Moreover, NGF expression could be a good target for treatment of the hyper-responsive intestine.

In conclusion, NGF overexpression regulates the nerve remodeling that modifies the sensitivity of intestinal motor reflexes. This remodeling affects afferent as well as motor innervation. The similarity between this experimental model of hypermotility and clinical intestinal motor syndromes, such as IBS, allows us to suggest anti-NGF strategies as a treatment for these diseases.

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References


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