Alterations of intestinal motor responses to various stimuli after Nippostrongylus brasiliensis infection in rats: role of mast cells

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Abstract Nippostrongylus brasiliensis infection induces jejunal mastocytosis associated with enteric nerve remodelling in rats. The aim of this study was to evaluate the intestinal motility responses to meals and to neurotransmitters involved in the control of gut motility (acetylcholine (carbachol), substance P and neurokinin A) in both control and N. brasiliensis-infected rats 30 days post-infection. All rats were equipped with NiCr electrodes in the jejunum to record myoelectrical activity. The duration of disruption of the jejunal migrating myoelectrical complexes (MMC) induced by the different stimuli was determined. Meal ingestion and substance P administration disrupted the MMC pattern for similar durations in the two groups. Carbachol and neurokinin A induced a significantly longer MMC disruption in post-infected rats than in controls (125 ± 8.3 vs. 70 ± 6 min for carbachol 100 µg kg⁻¹ and 51 ± 4 vs. 40 ± 2 for neurokinin A 50 µg kg⁻¹). The enhanced motor response in postinfected rats was reduced by previous mast cell stabilization with ketotifen or mast cell degranulation with compound BrX 537 A. In conclusion, the increased intestinal motor reactivity in basal conditions, after a meal and after administration of carbachol, substance P or neurokinin A in post-N. brasiliensis-infected rats depends upon intestinal mast cell hyperplasia and degranulation.

Keywords carbachol, jejenum, motility, Nippostrongylus brasiliensis, rat, tachykinins.

INTRODUCTION

Mast cells play a critical role in gastrointestinal neuroimmune interactions and their pathophysiological importance has been underlined.¹ For example, mast cell hyperplasia has been described in the terminal ileum of irritable bowel syndrome (IBS) patients.² Because of the close proximity between mast cells and sensory nerve fibres in the intestine,³ a role for mast cells has been suggested in the alterations of both intestinal motility and visceral sensitivity observed in IBS.²

It has recently been shown that the jejunal mast cell hyperplasia observed in rats after infection with the nematode Nippostrongylus brasiliensis (N. brasiliensis) is associated with a jejunum-specific hypersensitivity to distension involving mast cell degranulation, as the effect was reduced by the mast cell stabilizer doxantrazole.⁴ However, there are no experimental data to reinforce the hypothesis of a role for mast cells in the alteration of intestinal motility described in IBS.² Data concerning alterations of basal intestinal motility in IBS are very controversial. In contrast, several studies show that patients with IBS have increased motility in response to enteric stimuli such as meals,⁵ balloon inflation,⁶ or cholecystokinin.⁷ Consequently, this study uses the post-N. brasiliensis-infection model in rats to determine whether intestinal mastocytosis may induce alterations of intestinal motility. We investigated therefore intestinal motility changes induced by mastocytosis in basal conditions, after a meal and after administration of carbachol, substance P or neurokinin A. These two neuropeptides have been selected as it has been shown that among the long-term effects of nematode infection there is an increase in intestinal substance P concentration and a shift towards a tachykininergic regulation of the gut.⁸
MATERIALS AND METHODS

Animal preparation
Experiments were performed in control and post-\textit{N. brasiliensis}-infected male Wistar rats (300–350 g). Infection was achieved via subcutaneous (s.c.) injection of 3000 third-stage infective larvae of \textit{N. brasiliensis} in 0.5 mL sterile saline into the flank of the rats. Controls received a single s.c. injection of 0.5 mL sterile saline. Both dose and route of administration of infective larvae were consistent with previous studies. Post-infected rats were used 30 days after larvae administration. This period was selected because the parasite was eliminated by day 20 post-infection and at 30 days acute inflammation was resolved while mast cell hyperplasia was still present.

Rats were individually housed, fed a standard pelleted diet (A 04; U.A.R, Epinay-sur-Orge, France) and had free access to water. They were prepared for long-term electromyographic recordings of intestinal motility using a previously described technique. Briefly, under acepromazine (Vétocom, Lure, France) (0.5 mg kg$^{-1}$) and ketamine (Rhoˆne-Mérieux, Lyon, France) (100 mg kg$^{-1}$) anaesthesia, NiCr wire electrodes, 80 μm in diameter (Driver-Harris, Mantes la Jolie, France) were implanted in the jejunum (5, 15 and 25 cm aboral to the ligament of Treitz). The electrode wires were exteriorized on the back of the neck and protected by a glass tube attached to the skin. All protocols were approved by the Local INRA Animal Care and Use Committee.

Motility recordings
Electromyographic recordings began 7 days after surgery. Spiking activity was recorded with an electroencephalograph (MiniHuit; Alvar, Paris, France) with a paper speed of 3.6 cm min$^{-1}$, summed every 20 s by an integrator circuit, and automatically plotted 24 h per day on the y-axis of a potentiometric recorder with a paper speed of 5 cm h$^{-1}$. This integrated record permitted a clear determination of interdigestive migrating myoelectrical complexes (MMC) and postprandial patterns of myoelectrical activity at jejunal level.

Experimental procedures
The same treatments were applied to controls and post-infected rats, and motility parameters (frequency and velocity of propagation of MMC, duration of the disruption of MMC after stimuli) were compared between the two groups of animals.

In a first series of experiments, in 14 h-fasted rats, when the MMC pattern displayed a clear fasted pattern, frequency and velocity of propagation of MMC were compared between controls ($n=8$) and post-infected rats ($n=8$).

In a second series of experiments, in 14 h-fasted rats, when the myoelectrical activity of the jejunal displayed an organized MMC pattern, a postprandial pattern of intestinal motility was induced by ingestion of a solid meal (3 g of pelleted food). However, because of possible differences in the ingestion rates of controls ($n=7$) and post-infected rats ($n=7$) which could influence the motor response to the meal, postprandial motility was also induced by a liquid meal (0.66 g of powdered whole cow’s milk dissolved in 2 mL of water) given by oro-gastric gavage ($n=8$ for both controls and post-infected rats).

In a third series of experiments, we determined the effect of a nonhydrolysable cholinomimetic agonist (carbachol, 100 μg kg$^{-1}$ s.c.) on intestinal motility by measuring the duration of disruption of the MMC pattern in controls ($n=10$) and post-infected rats ($n=10$).

In a fourth series of experiments, we assessed the effect of a tachykininergic stimulation on intestinal motility by giving either substance P (200 μg kg$^{-1}$ i.p., $n=8$ for both controls and post-infected rats) or neuropeptide A (20 and 50 μg kg$^{-1}$ i.p., $n=8$ for both controls and post-infected rats).

Finally, when significant differences in intestinal motility were noticed between controls and post-infected rats, the same experiments were repeated after previous treatment with a mast cell degranulator (BrX 537 A, 2 mg kg$^{-1}$ i.p.) or a mast cell stabilizer (ketotifen, 1 mg kg$^{-1}$ i.p.) to evaluate the possible contribution of mast cells. Rats received two injections of BrX 537 A, at a 14 h interval, the last one being performed $2$ h before the administration of carbachol (100 μg kg$^{-1}$ s.c., $n=8$ for both controls and post-infected rats) or neuropeptide A (50 μg kg$^{-1}$ i.p., $n=8$ for both controls and post-infected rats). Degranulation of mast cells by BrX 537 A is known to disrupt MMC for about 1 h and the delay of 2 h permitted the occurrence of MMC at the time of administration of either carbachol or neuropeptide A. Two other groups of rats were given ketotifen twice daily for 4 days, and received the last administration 2 h before either carbachol or neuropeptide A.

Histological study
Three groups of post-\textit{N. brasiliensis}-infected rats, 30 ($n=6$), 60 ($n=5$) and 90 ($n=6$) days post-infection,
were used for mast cell counts. A fourth group of rats \((n = 4)\), used as control, received a s.c. injection of 0.5 mL sterile saline 30 days before sacrifice. Rats were killed by cervical dislocation and exsanguination. Small pieces (5 mm) of jejunum, 10 cm distal to the ligament of Treitz, and proximal colon, 3 cm from the caecocolonic junction, were fixed in Carnoy’s solution, cleared in toluene and embedded in paraffin blocks. Transverse sections \((5 \mu m)\) were stained with alcian blue-safranin O according to Roberts et al.,\(^{13}\) for identification of intestinal mast cells, both in the mucosa and submucosa. Three sections per animal and three views per section were examined for mast cell counts. The sections were examined in a blind fashion.

**Drugs**

BrX 537 A (bromolasalocid ethanolate) was a gift from Roche Laboratories [London, England]. It was dissolved in dimethylsulfoxide (DMSO). The dose of BrX 537 A used \((2 \text{ mg kg}^{-1})\) has been found to degranulate mast cells in a reproducible manner. A study by Castex et al.,\(^{14}\) has shown that the number of granulated mast cells was significantly \((P < 0.001)\) reduced in the mucosa and submucosa 1 h and 5 h after administration of BrX 537 A at a dose of 2 mg kg\(^{-1}\). Ketotifen (fumarate salt) was obtained from Sigma Chemical Company [St Louis, MO, USA] and was dissolved in sterile saline. A previous study showed that the dose of ketotifen used in this study \((1 \text{ mg kg}^{-1} \text{ twice daily for 4 days})\) prevented the release of mast cell mediators induced by Clostridium difficile toxin A.\(^{15}\)

**Data analysis**

The duration of MMC disruption after different treatments was expressed as the mean ± SEM. Statistical analysis was performed using Student’s t-test for unpaired data or Mann–Whitney test where appropriate. The number of mast cells per square millimeter of jejunal and proximal colonic mucosa and submucosa was counted with an image grabbing programme [Neotech, Paris, France] and an Optilab Pro image analysis software package [Graftek, Paris, France] running on Apple Macintosh 7100/80 computer [Cupertino, CA, USA]. The number of mast cells in the different groups of animals was expressed as the mean ± SEM and compared with the Kruskal–Wallis nonparametric test followed by the Mann–Whitney test. \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Tissue histology**

Inflammatory sites were not detected macroscopically or microscopically in intestinal samples from both controls and post-\(N. \text{brasiliensis}\)-infected rats, 30, 60 and 90 days post-infection. Post-infected rats were characterized by jejunal mastocytosis persisting until day 90 after infection. The number of mucosal mast cells in the jejunum 30 days postinfection was four times higher in post-infected rats \((597 ± 91 \text{ mast cells mm}^{-2}, n = 6)\) than in controls \((151 ± 22 \text{ mast cells mm}^{-2}, n = 4)\). At days 60 and 90 post-infection, the number of mast cells was reduced in comparison with day 30 but remained significantly higher \((P < 0.05)\) than in controls.

The number of mast cells in the proximal colon was \(126 ± 15 \text{ mm}^{-2}\) in controls and was not significantly modified on days 30, 60 and 90 after infection (Fig. 1).

**Motility studies**

**Fasted pattern** The frequency and velocity of propagation of MMC were, respectively, \(3.9 ± 1 \text{ MMC h}^{-1}\) and \(1.24 ± 0.09 \text{ cm min}^{-1}\) in controls and were not significantly different in post-infected rats \((4.0 ± 1.1 \text{ MMC h}^{-1} \text{ and } 1.30 ± 0.07 \text{ cm min}^{-1})\).

**Postprandial motility** Postprandial MMC disruption after a 3-g solid meal lasted \(143.0 ± 27.4 \text{ min}\) in controls and was not significantly modified on days 30, 60 and 90 after infection (Fig. 1).

**Figure 1** Number of mucosal mast cells in the jejunum and proximal colon of controls and 30, 60, 90 days post-\(N. \text{brasiliensis}\)-infected rats. *significantly different \((P < 0.05)\) from controls.
controls and was not significantly different in post-infected rats \(184.7 \pm 31.7 \text{ min}\). Similarly, the duration of MMC disruption after intragastric administration of the liquid meal \(2 \text{ mL}\) was not significantly different in both controls and post-infected rats \(77.1 \pm 12.6 \text{ and } 74.5 \pm 5 \text{ min, respectively}\) [Fig. 2].

**Cholinergic stimulation** Carbachol \(100 \text{ µg kg}^{-1} \text{ s.c.}\) disrupted the MMC pattern that was replaced by an intense irregular activity for \(70.0 \pm 5.5 \text{ min}\) in controls and \(125.2 \pm 8.3 \text{ min}\) in post-infected rats \(P < 0.0001\) [Fig. 3]. The mast cell stabilizing agent ketotifen \((1 \text{ mg kg}^{-1}, 4 \text{ days, twice daily})\) did not modify significantly the MMC disruption induced by carbachol in controls \(74.4 \pm 3.9 \text{ min}\) but reduced the effect in post-infected rats \(105.9 \pm 6.0 \text{ min, } P < 0.05\). Similarly, previous administration of the mast cell degranulator BrX 537 A \(2 \text{ mg kg}^{-1}, \text{ twice}\) did not modify significantly the duration of MMC disruption induced by carbachol in controls \(72.6 \pm 6.5 \text{ min}\) but significantly reduced the disruption in post-infected rats \(80.7 \pm 3.2 \text{ min, } P < 0.0001\) [Fig. 4].

**Tachykininergic stimulation** Substance P \(200 \text{ µg kg}^{-1} \text{ i.p.}\) disrupted the MMC pattern for \(28.1 \pm 2.8 \text{ min in}\)

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![Figure 2](image2.png) **Figure 2** Duration of the disruption of jejunal MMC after a solid or a liquid meal in controls and 30 days post-\(N.\ brasiliensis\)-infected rats. No significant difference \(P > 0.05\) was observed between the two groups of animals.

![Figure 3](image3.png) **Figure 3** Representative integrated jejunal electromyograms obtained in control and post-\(N.\ brasiliensis\)-infected animals after s.c. administration of carbachol \((100 \text{ µg kg}^{-1})\). Duration of the MMC disruption is significantly enhanced in post-\(N.\ brasiliensis\)-infected rats.
controls. This duration was not significantly different in post-infected rats [22.5 ± 2.8 min].

In controls, neurokinin A, at the doses of 20 and 50 μg kg⁻¹, disrupted the MMC for 31.2 ± 1.8 and 40.1 ± 1.8 min, respectively. These durations were significantly (P < 0.05) increased (44.4 ± 5 and 51.6 ± 3.8 min, respectively) in post-infected rats (Fig. 5a). Ketotifen [1 mg kg⁻¹, 4 days, twice daily] did not modify the duration of MMC disruption caused by neurokinin A [50 μg kg⁻¹] in controls but reduced it significantly (P < 0.05) in post-infected rats. BrX 537 A prevented the increase in the duration of MMC disruption observed in post-infected rats and reduced significantly (P < 0.05) this duration in controls (Fig. 5b).

DISCUSSION

Nippostrongylus brasiliensis infection in rats is widely used as a model for studying pathological conditions of the intestine such as inflammation or antigenic sensitization. This nematode infests the proximal small intestine where it produces an early acute inflammation which peaks 2 weeks after infection. At this time, the worms are spontaneously eliminated and mastocytosis develops in the mucosa. One month after infection, inflammation has resolved while mucosal mast cell hyperplasia is still present and remains for several weeks. Using the N. brasiliensis post-infection model, our results indicate that, in the absence of inflammation, jejunal mastocytosis is not associated with disturbances of intestinal motility in basal conditions but may trigger exaggerated responses to some stimuli.

The absence of jejunal motor alterations in the fasted state in post-N. brasiliensis-infected rats has been already reported: the MMC pattern has been found to be markedly disrupted from day 5 to day 10 after...
infection while normal MMCs were recovered three weeks after infection. Nevertheless, there is no evident reason for an alteration of intestinal motility associated with mastocytosis in the absence of any stimulation inducing mast cell degranulation and subsequent release of mediators which may affect intestinal motility.

Mast cell degranulation by BrX 537 A or by antigenic challenge in ovalbumin-sensitized rats is known to disrupt the MMCs and to induce a motility pattern similar to that observed after a meal. In a recent study, it has been shown, first, that meal-induced cholecystokinin (CCK) release is able to degranulate mucosal mast cells and, second, that duodenal infusion of nutrients (ovalbumin hydrolysate) induces mast cell degranulation through the release of CCK. Another postprandial hormone, gastrin, has also been shown to stimulate gastric mast cells. Consequently, one could expect meal ingestion to induce stronger motor intestinal effects in the presence of an intestinal mastocytosis. In fact, we observed no difference in the duration of the postprandial disruption of MMC between control and post-infected rats, whatever the nature of the meal (either solid spontaneously eaten or liquid intragastrically administered). We may suppose that CCK release induced by the meals is not sufficient to induce mast cell degranulation or that intestinal mast cells of post-infected rats are less sensitive to CCK than those of control rats.

Our results indicate that carbachol induces a longer jejunal motor response in post-\(N.\) brasiliensis-infected rats than in controls. This is in agreement with the altered cholinergic neurotransmission observed several days after the intestinal inflammation induced by \(Trichinella spiralis\) in mice and ferrets has resolved. This increased response very likely involves mast cell degranulation as it was reduced by stabilization of mast cells with ketotifen and suppressed by previous depletion of mast cells by BrX 537 A. The fact that stabilization or depletion of mast cells modified the response to carbachol in post-infected but not in control rats reinforces the concept of functional differences between the intestinal mast cells of naïve rats and those resulting from the hyperplasia associated with nematode infection. For example, it has been shown that in \(N.\) brasiliensis-infected mice, the expression of house mast cell protease-1 is up-regulated and that in \(N.\) brasiliensis-infected rats, the expression of the mast cell receptor Fe-epsilon is increased. Several hypotheses can explain the enhanced response to carbachol in post-infected rats in comparison with controls. It is known that \(N.\) brasiliensis produces very large amounts of acetylcholinesterase which has been found to modulate the proliferation of epithelial cells. We can suppose that the subsequent decrease in acetylcholine availability induces an up-regulation of muscarinic receptors which would explain the increased reactivity to carbachol. It has already been shown that the \textit{in vitro} jejunal contraction induced by carbachol is greater in \(N.\) brasiliensis-infected rats than in controls while the muscarinic receptor binding characteristics are not different between the two groups. However, it is difficult to compare these results with ours as the binding study was performed on smooth muscle cells, but not on mast cells and the results were obtained in the early period after infection (8 days). The greater response to carbachol can also reflect some direct effect of the inflammatory response to the worms on cholinergic nerve function which is not necessarily driven by the worm and does not involve changes in the number of muscarinic receptors. This enhanced response can be a consequence of the profound nerve remodelling associated with mast cell hyperplasia as a large number of neural processes closely associated with mast cells have been identified as cholinergic neurones. Nevertheless, whatever the mechanisms involved, mast cell stimulation by cholinomimetic compounds is well documented and most studies suggest the presence of muscarinic receptors on mast cells.

In the last part of our study we showed that the intestinal motor response to neurokinin A, but not to substance P, was longer in post-infected rats than in controls. The absence of difference in the effect of substance P was unexpected as mast cell degranulation by substance P is well documented (for review, see Maggi et al., 1997). Moreover, the number of substance P-immunoreactive nerve fibres in the jejunal mucosa 35 days after \(N.\) brasiliensis infection was increased to approximately 150% of control values and the intestinal content of substance P was also increased to 150% by day 10, and returned to control values by day 35. Lack of difference in the effects of substance P cannot be attributed to an absence of reactivity of the new mucosal mast cells resulting from the hyperplasia as it has been shown that these cells release histamine when challenged with substance P. However, most data showing mast cell degranulation by substance P were obtained \textit{in vivo}, with isolated mast cells or tissue strips, at relatively high concentrations of substance P, in the \(\mu M\) range (for review, see Maggi et al., 1997). Thus, the dose which disrupted MMCs in our study may not be sufficient to degranulate mast cells. On the other hand, the enhanced action of neurokinin A agrees with previous results obtained with the same model.
of mastocytosis which showed greater gastric or jejunal hyperaemia, mediated through mast cell degranulation, after application of capsaicin, which induced tachykinin and calcitonin gene-related peptide release from sensory afferents.\textsuperscript{36,37} We showed that the enhanced effect of neurokinin A in post-infected rats depends upon mast cell degranulation as it was reduced by BrX 537 A and abolished by ketotifen pretreatments, in agreement with several studies showing that neurokinin A is able to degranulate mast cells.\textsuperscript{38} Moreover, the disruption of MMC in control rats also very likely involves mast cell degranulation as it was reduced by a previous depletion of mast cell contents by BrX 537 A. However, pretreatment with the mast cell stabilizer ketotifen was not able to reduce the effects of neurokinin A in control rats. This would mean that ketotifen had a greater efficacy to stabilize mast cells which develop after worm infection than original mast cells. Such a hypothesis is consistent with the fact that ketotifen is not able to stabilize all types of mast cells; for example, ketotifen inhibits the IgE-dependent release from lung and tonsilar mast cells but not from skin mast cells.\textsuperscript{39}

Finally, besides the enhanced nociceptive response to intestinal distension already shown,\textsuperscript{4} our results indicate that the intestinal mast cell hyperplasia and the nerve remodelling induced by nematode infection are associated with an increased intestinal motor response to excitatory agonists such as acetylcholine or neurokinin A.

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