Inhibition of acetylcholine induced intestinal motility by interleukin 1β in the rat

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Abstract

**Background/Aims**—The fact that raised interleukin 1β (IL1β) concentrations have been found in the colonic mucosa of rats with experimentally induced colitis and of patients with inflammatory bowel disease indicates that this cytokine may participate in the disturbed intestinal motility seen during inflammatory bowel disease. This study investigated whether IL1β could change the contractility of (a) a longitudinal muscle–myenteric plexus preparation from rat jejunum, ileum, and colon and (b) isolated jejunal smooth muscle cells.

**Methods**—Isometric mechanical activity of intestinal segments was recorded using a force transducer. Moreover, smooth muscle cell length was measured by image analysis.

**Results**—Although IL1β did not affect jejunal, ileal, and colonic basal contractility, it significantly reduced contractile response to acetylcholine (ACh). This significant inhibition was seen only after 90 or 150 minutes of incubation with IL1β. Pretreatment with cycloheximide blocked IL1β induced inhibition of ACh stimulated jejunal contraction, suggesting that a newly synthesised protein was involved in the effect. Nω-nitro-L-arginine (a nitric oxide synthase inhibitor) did not prevent the inhibition induced by IL1β. Blocking neural transmission with tetrodotoxin abolished the IL1β effect on jejunal contractile activity, whereas IL1β had no effect on isolated and dispersed smooth muscle cells.

**Conclusions**—IL1β inhibits ACh induced intestinal contraction and this inhibitory effect involves protein synthesis but is independent of nitric oxide synthesis. This effect does not involve a myogenic mechanism but is mediated through the myenteric plexus.

**Keywords:** interleukin 1β, intestinal motility, enteric nervous system, rat.

Interleukin 1β (IL1β) is a pro-inflammatory protein produced by various cell types, including monocytes, platelets, chondrocytes, fibroblasts, keratinocytes, endothelial cells, and smooth muscle cells. It is one of the key mediators involved in inflammatory reactions. Significantly increased concentrations of IL1β have been found in the distal colonic mucosa of rats with experimentally induced colitis and in the intestine of patients with Crohn's disease. Inflammatory intestinal disease in humans or animals is accompanied by motility changes, which may reflect alteration in the function of smooth muscle or the enteric nervous system, or both. IL1β can also modulate the release of acetylcholine, norepinephrine, and substance P, which are neuromediators located in the rat myenteric plexus. Moreover, change in the colonic myoelectrical activity after induction of colitis with trinitrobenzene sulfonic acid was significantly reduced by an interleukin 1 receptor antagonist (IL1-ra). These findings suggest a role for endogenous IL1β as a mediator of changed neural function or motor activity in the inflamed intestine.

Few data concerning the effects of IL1β on intestinal motility are available in published reports, and its mechanism of action is not completely understood. In vivo, the central stimulatory action of endogenous IL1β on intestinal motility seems to involve endogenous prostaglandins. Conversely, the action of IL1β on gastric motility is inhibitory. Indeed, both central and systemic administrations of this cytokine induce a long-lasting delay in rat gastric emptying, an action that is mediated through central IL1 receptors. The purpose of this study was to determine the effect of IL1β on rat intestinal contractility in vitro and to investigate its mechanism of action more thoroughly.

**Methods**

**MEASUREMENT OF MUSCLE CONTRACTION**

**Animals and apparatus**

Male Wistar rats (250–300 g) were killed by cervical dislocation. One to 1.5 cm long segments of jejunum, ileum, and proximal colon were quickly removed, opened along the mesenteric border, and cleaned of intraluminal contents in a Krebs-bicarbonate solution (pH 7.4) composed of (mmol/l): 128 NaCl, 4.5 KCl, 2.5 CaCl2, 1.18 MgSO4, 1.18 KH2PO4, 125 NaHCO3, and 5.55 D-glucose. Longitudinal muscle–myenteric plexus (LM-MP) preparations from jejunum, ileum, and colon were peeled from the underlying circular muscle and then suspended under 1 g (for jejunum and ileum) or 2 g (for colon) of tension in a 1 ml organ bath containing continuously oxygenated (5% CO2, 95% O2) Krebs-bicarbonate solution. Preparations were then allowed to...
equilibrate for 60 minutes. The isometric longitudinal mechanical activity of the segments was recorded using a force transducer (Basile no 7005, Comerio, VA, Italy), as previously described. At the beginning of each experiment, acetylcholine chloride (ACh, 10^{-3} M) was applied and served as a control. A complete dose response curve to ACh (10^{-6} to 10^{-3} M) was also established. Test substances were applied successively with repeated washings of 20 to 30 minutes between each concentration. The viability of each preparation was checked at the end of each experiment by controlling spontaneous mechanical activity and the response to 10^{-5} M ACh. Results were expressed as tension (g) and normalised for cross sectional area (CS), which was determined using the following equation:

\[ \text{CS (mm)}^2 = \left( \frac{\text{tissue wet weight (mg)}}{\text{tissue length (mm)}} \right) \times \text{density (mg/mm)}^3 \]

A density of 1.05 was used according to Vermillon et al.

Experimental design
In a first set of experiments, the effect of IL1β was studied on spontaneous and ACh induced contractility. ACh (10^{-3} M) was applied on the LM-MP jejunal preparation five, 90 or 150 minutes after incubation with IL1β (1-50 ng/ml). A dose response curve to ACh (10^{-6} to 10^{-3} M) was obtained in a cumulative manner after 90 minutes incubation with IL1β (10 ng/ml). ACh (10^{-3} M) was also applied on LM-MP ileal and colonic preparations five or 90 minutes after incubation with IL1β (10 ng/ml). To exclude an effect caused by possible endotoxin contamination, IL1β (10 ng/ml) was boiled for 20 minutes before being added to the tissue. In a second set of experiments, the effect of potential inhibitory agents on the LM-MP jejunal motor response to ACh (10^{-5} M) was investigated before and after 90 minutes exposure to IL1β (10 ng/ml). The following drugs were used: cycloheximide (3-5×10^{-4} M) to inhibit protein synthesis, Nω-nitro-L-arginine (L-NNA, 3×10^{-4} M) to inhibit nitric oxide synthase, and tetrodotoxin (TTX, 10^{-4} M) to block nervous transmission. L-NNA and cycloheximide were applied 15 and 20 minutes respectively before IL1β; and TTX was added to the bath five minutes before application of IL1β. In control experiments, a volume of Krebs-bicarbonate solution equal to the volume of the inhibitory agents used was added to the bath. All tested substances were administered in a volume that did not exceed 1% of the whole bath volume.

Measurement of contractile response in dispersed smooth muscle cells

Cell preparation
Smooth muscle cells were isolated from LM-MP of rat jejunum using a modification of the method of Bitar et al., as previously described. The LM-MP preparation was cut into small pieces and incubated at 37°C for three successive 30 minute periods in Ca^{2+}-free phosphate buffer solution (PBS) containing 2% bovine serum albumin, 0.1% collagenase, and 0.1% soybean trypsin inhibitor. The pieces were washed in enzyme free PBS, and the cells were allowed to disperse under gentle pipette trituration.

Measurement of contractile response
Cells (10^5) were exposed to ACh (10^{-7} M) for three minutes before being fixed with 3% glutaraldehyde. Cell length was measured by image analysis using Image 1.49 software (NIH, Bethesda, MD, USA). The length of 50 cells was measured in each experiment, and the results expressed as mean (SEM). To test the effect of IL1β on ACh induced contraction, cells were incubated for 90 minutes with PBS (control experiments) or IL1β (10 ng/ml) before being exposed to ACh (10^{-7} M).

Data analysis
All data are presented as means (SEM). Significance among groups was tested by one way analysis of variance (ANOVA).

Chemicals
Drugs and chemicals were purchased from Sigma Chemical Co (L’Isle d’Abeau Chesnes, St Quentin Fallavier Cedex, France), and enzymes from ICN (Orsay, France). Human recombinant IL1β was obtained from Pepro Tech (Le Perray en Yvelines, Paris, France).

Results

Effects of IL1β on LM-MP preparations
IL1β (1 to 50 ng/ml) applied to jejunal LM-MP preparations over a period of five, 90 or 150 minutes did not affect the basal tone of jejunal muscle. In the same way, IL1β (10 ng/ml) applied to ileal and colonic LM-MP preparations over five or 90 minutes did not change the basal tone of intestinal muscle (data not shown).

Addition of IL1β to the bath five minutes before ACh did not modify the ACh evoked contractile intestinal response. However, exposure of the jejunal preparation to IL1β (10 or 50 ng/ml) for 90 or 150 minutes resulted in a significant (p<0.05, n=8) reduction of ACh induced contraction. Inhibition after 90 and 150 minutes incubation with 10 ng/ml of IL1β was 23+1 (3%) and 30-1 (10%) respectively (Fig 1A and 1B). A dose response curve to ACh was performed after 90 minutes exposure to IL1β (10 ng/ml) showing that the inhibitory effect of IL1β was maintained whatever the ACh concentration used (Fig 2). Exposure of ileal and colonic segments to IL1β (10 ng/ml) for 90 minutes significantly reduced ACh induced contraction (Fig 3A and 3B).

Preincubation of the LM-MP preparations with 10 ng/ml of boiled IL1β did not affect the ACh (10^{-5} M) induced jejunal response (Fig 1A).

The inhibiting action of IL1β was completely abolished by cycloheximide (3.5×10^{-4}}
Figure 1: Effect of interleukin 1β (IL1β, 1, 10 and 50 ng/ml) added to the bath 90 minutes (A) and 150 minutes (B) before 10⁻⁷ M acetylcholine (ACh) induced contractions in isolated longitudinal muscle–myenteric plexus preparation of rat jejunum. Effect of boiled IL1β on the ACh induced jejunal response (A). The viability of the preparation was assessed by their response to ACH at the end of each experiment (A), (B). Each column represents the mean (SEM) of values obtained from 12 strips. *p<0.05 versus ACh alone.

Figure 2: Effect of interleukin 1β (IL1β: 10 ng/ml) added to the bath 90 minutes before acetylcholine (ACh) dose response curve (10⁻⁷ M to 10⁻⁴ M) in isolated longitudinal muscle–myenteric plexus preparation of rat jejunum. Each point represents the mean (SEM) of values obtained from eight strips. *p<0.05 versus ACh alone.

M), which however did not modify the contractile effect of ACh when given alone (Fig 4).

The nitric oxide synthase inhibitor L-NNA (3×10⁻⁴ M) did not prevent the inhibition caused by IL1β but increased the ACh response of smooth muscle when given alone (Fig 4).

Blockade of neural transmission by TTX (10⁻⁶ M) suppressed the inhibition of ACh induced contraction caused by IL1β while TTX alone did not alter ACh induced jejunal contraction.

Figure 3: Inhibitory effect of interleukin 1β (IL1β) added to the bath 90 minutes before 10⁻⁷ M acetylcholine (ACh) induced contractions in isolated longitudinal muscle–myenteric plexus preparation of rat ileum (A) and colon (B). Each column represents the mean (SEM) of values obtained from 12 strips. *p<0.05 versus ACh alone.

EFFECT OF IL1β ON CONTRACTION OF DISPERSED JEJUNAL SMOOTH MUSCLE CELLS
Isolated cells of various lengths (range 26 to 83 μm) were found in the unstimulated preparation. Exposure of smooth muscle cells to IL1β (10 ng/ml) for 90 minutes had no significant effect on their length (52.7 (1.9) versus 55.6 (2.3) μm). Application of ACh (10⁻⁷ M) on jejunal smooth muscle cells after 90 minutes of incubation in PBS resulted in a significant cell shortening of 23.5 (6)% (p<0.001). When smooth muscle cells were preincubated with IL1β for 90 minutes, the contractile effect of
ACh did not change significantly (40.3 (1.4) versus 42.3 (1.8) μm).

Discussion

The results of this investigation show that IL1β is a potent inhibitor of rat jejunal, ileal, and colonic smooth muscle contraction in response to ACh. This effect was only apparent after a certain delay (90 or 150 minutes) after initial exposure to IL1β. The effect on jejunum was abolished by cycloheximide and TTX but persisted after pretreatment with L-NNA. The potent inhibitory motor effect of IL1β seen in our experimental conditions was not caused by contamination by endotoxin (for example, lipopolysaccharide) because it was heat sensitive. The ability of IL1β to inhibit ACh induced jejunal contraction corroborates previous reports showing that IL1β inhibits the in vitro motility of gastric and other smooth muscles, including vascular and airway ones.16 17

As inflammatory bowel disease occurs mainly in terminal ileum and right colon, we evaluated the effect of IL1β on ACh ileal and proximal colonic contractile response. Our in vitro findings indicate that IL1β (10 ng/ml), as seen for the jejenum, produced a similar inhibitory effect in rat ileum and colon. A recent in vivo study found that IL1β affected the motility of various regions of rat ileum and colon. The notion that IL1β suppresses postprandial motility at the jejunal site and modulating caecocolic motor activity in conscious rats.9

As reported in previous studies performed on vascular and airway smooth muscle,16 17 the inhibitory effect of IL1β on jejunal motility was found to be concentration dependent. The IL1β concentrations that were effective in our study are similar to those that inhibited gastric motility10 and ACh release from rat longitudinal muscle preparation.9 This concentration range is similar to that measured in fresh intestinal mucosa of patients with IBD.18

Significantly more IL1β is produced spontaneously by mononuclear cells from inflamed than normal colonic mucosa.19 As colonic motor abnormalities observed in experimental models of colitis were reduced by administration of IL1-ra,2 it is probable that IL1β acts as a modulator of motility in both healthy and diseased intestine.

IL1β induced inhibition of jejunal contractility occurred only 90 or 150 minutes after exposure to the cytokine, which is in agreement with reports showing that the effect of IL1β on vascular and airway smooth muscle is time dependent.16 17 The delayed action of IL1β suggests a mechanism involving the synthesis of a new molecule, which may well be a protein. As in the study of Main et al28 concerning the effect of IL1β on ACh release, we found that cycloheximide blocked the IL1β effect on intestinal motility completely, suggesting that a newly synthesised protein was involved.

Nitric oxide synthase could be the intermediate protein involved in the IL1β effect. This enzyme has been found in vascular smooth muscle29 and in myenteric plexus.30 It is involved in the synthesis of nitric oxide, which is a potent vasorelaxant mediator and an inhibitory enteric neurotransmitter in the gastrointestinal tract. Beasley et al31 found that the IL1β induced relaxant effect on vascular contraction in the rat was blocked by a potent inhibitor of nitric oxide synthase (N(G)-monomethyl-L-arginine). However, in our study, application of L-NNA failed to inhibit the action of IL1β. Our findings are consistent with other findings in the rat in which nitric oxide synthase blockade did not change IL1β induced relaxation of the proximal stomach.32 It is noteworthy that pretreatment with L-NNA alone significantly increased the ACh induced contractile response. This finding corroborates earlier findings indicating that inhibition of nitric oxide synthase, through blockage of the synthesis of the relaxant nitric oxide, increases electrically induced cholinergic contractions of guinea pig ileum and taenia coli.33 24

The fact that the contractile inhibitory effect of IL1β was abrogated by prior administration of TTX strongly suggests the existence of a neural mediation pathway. Such mediation has also been suggested in IL1β induced colonic hypersecretion in the rat.33 Moreover, it has been shown that exogenous IL1β inhibits both ACh and norepinephrine release from rat myenteric plexus.34 The notion that IL1β receptors exist on the neurons of the myenteric plexus is indirectly supported by autoradiographic studies showing that IL1β receptors occur on neurons of the central nervous system.35 Other studies have shown that IL1β can modulate invertebrate neuron functions,36 and it has been postulated that this cytokine acts directly on the mammalian brain.31 Finally, myenteric plexus would seem to be the target of IL1β as this cytokine had no effect on isolated dispersed smooth muscle cells in our study or in airway smooth muscle.17

Although the nature of the protein potentially involved with IL1β has not been determined, IL1β might release other cytokines, including interleukin 6 (IL6) and tumour necrosis factor,25 26 which could in turn affect gastrointestinal motility.10 In fact, it has been shown that IL1β induces IL6 expression in rat intestinal smooth muscle cells.35 Another peptide mediating IL1β induced inhibition of contraction may be the vasoactive intestinal peptide, a major non-cholinergic non-adrenergic inhibitory
transmitter of intestinal contraction. We are currently testing this hypothesis by using a vaso-active intestinal peptide receptor antagonist.

We can exclude the possibility that the inhibitory effect of ILβ reflect changes in the acetylcholinesterase activity (enzyme which degrades ACh) and therefore in the bioavailability of ACh in presence of ILβ. In fact, application of neostigmine, an acetylcholinesterase inhibitor, failed to affect the inhibitory action of ILβ data not shown). Our finding corroborates earlier findings indicating that ILβ suppresses ACh release in LM-MP jejunal preparations superfused with Krebs' buffer solution containing physostigmine, an acetylcholinesterase inhibitor, suggesting that the ILβ inhibitory effect could remain despite the inhibition of acetylcholinesterase. Moreover, the inhibitory effect of ILβ was maintained even at high concentration. Finally, Palmer and Koch have recently shown that jejunal acute inflammation was associated with a decrease in acetylcholinesterase activity. Our study showed that ILβ inhibits the contractile response of rat jejunum, ileum, and colonic smooth muscle to ACh. Inhibition of jejunal contractility does not involve a myogenic mechanism but is mediated through the enteric nervous system. It is independent of nitric oxide synthesis but involves a newly synthesised protein. A previous study showed that the effect of ILβ on intestinal motility is mainly attributable to a central action. Our study suggests that a peripheral action may also be involved in the genesis of the intestinal motor alteration observed during inflammatory bowel disease.

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