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

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Abstract

Background/Aims—The fact that raised interleukin 1 β (IL1 β) 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 IL1 β 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 IL1 β 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 IL1 β . Pretreatment with cycloheximide blocked IL1 β induced inhibition of ACh stimulated jejunal contraction, suggesting that a newly synthesised protein was involved in the effect. N^w-nitro-L-arginine (a nitric oxide synthase inhibitor) did not prevent the inhibition induced by IL1 β . Blocking neural transmission with tetrodotoxin abolished the IL1 β effect on jejunal contractile activity, whereas IL1 β had no effect on isolated and dispersed smooth muscle cells.

Conclusions—IL1 β 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.

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

Interleukin 1 β (IL1 β) is a pro-inflammatory protein produced by various cell types, including monocytes, platelets, chondrocytes, fibroblasts, keratinocytes, endothelial cells, and smooth muscle cells.¹ It is one of the key mediators involved in inflammatory reactions. Significantly increased concentrations of IL1 β

have been found in the distal colonic mucosa of rats with experimentally induced colitis and in the intestine of patients with Crohn's disease.²⁻⁴ Intestinal inflammation 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.⁵ IL1 β can also modulate the release of acetylcholine, norepinephrine, and substance P, which are neuromediators located in the rat myenteric plexus.⁶⁻⁸ Moreover, change in the colonic myoelectrical activity after induction of colitis with trinitrobenzene sulphonic acid was significantly reduced by an interleukin 1 receptor antagonist (IL1-ra).² These findings suggest a role for endogenous IL1 β as a mediator of changed neural function or motor activity in the inflamed intestine.

Few data concerning the effects of IL1 β 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 IL1 β on intestinal motility seems to involve endogenous prostaglandins.⁹ Conversely, the action of IL1 β on gastric motility is inhibitory.¹⁰ Indeed, both central and systemic administrations of this cytokine induce a longlasting delay in rat gastric emptying, an action that is mediated through central IL1 receptors.¹¹ The purpose of this study was to determine the effect of IL1 β 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 10 ml organ bath containing continuously oxygenated (5% CO₂, 95% O₂) Krebs-bicarbonate solution. Preparations were then allowed to

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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.¹² At the beginning of each experiment, acetylcholine chloride (ACh, 10^{-5} M) was applied and served as a control. A complete dose response curve to ACh (10^{-9} 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 equation¹³:

$$CS (mm^2) = ((\text{tissue wet weight (mg)} / \text{tissue length (mm)}) \times \text{density (mg/mm}^3))$$

A density of 1.05 was used according to Vermillon *et al.*¹³

Experimental design

In a first set of experiments, the effect of IL1 β was studied on spontaneous and ACh induced contractility. ACh (10^{-5} M) was applied on the LM-MP jejunal preparation five, 90 or 150 minutes after incubation with IL1 β (1–50 ng/ml). A dose response curve to ACh (10^{-9} to 10^{-3} M) was obtained in a cumulative manner after 90 minutes incubation with IL1 β (10 ng/ml). ACh (10^{-5} M) was also applied on LM-MP ileal and colonic preparations five or 90 minutes after incubation with IL1 β (10 ng/ml). To exclude an effect caused by possible endotoxin contamination, IL1 β (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 IL1 β (10 ng/ml). The following drugs were used: cycloheximide (3.5×10^{-4} M) to inhibit protein synthesis, N w -nitro-L-arginine (L-NNA, 3×10^{-4} M) to inhibit nitric oxide synthase, and tetrodotoxin (TTX, 10^{-6} M) to block nervous transmission. L-NNA and cycloheximide were applied 15 and 20 minutes respectively before IL1 β ; and TTX was added to the bath five minutes before application of IL1 β . In control experiments, a volume of Krebs-bicarbonated 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.*¹⁴ as previously described.¹⁵ The LM-MP preparation was cut into small pieces and incubated at 37°C for three successive 30 minute periods in Ca²⁺-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 IL1 β on ACh induced contraction, cells were incubated for 90 minutes with PBS (control experiments) or IL1 β (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 IL1 β was obtained from Pepto Tech (Le Perray en Yvelines, Paris, France).

Results

EFFECTS OF IL1 β ON LM-MP PREPARATIONS

IL1 β (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, IL1 β (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 IL1 β to the bath five minutes before ACh did not modify the ACh evoked contractile intestinal response. However, exposure of the jejunal preparation to IL1 β (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 IL1 β 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 IL1 β (10 ng/ml) showing that the inhibitory effect of IL1 β was maintained whatever the ACh concentration used (Fig 2). Exposure of ileal and colonic segments to IL1 β (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 IL1 β did not affect the ACh (10^{-5} M) induced jejunal response (Fig 1A).

The inhibiting action of IL1 β was completely abolished by cycloheximide (3.5×10^{-4}

Figure 1: Effect of interleukin 1 β (IL1 β ; 1, 10 and 50 ng/ml) added to the bath 90 minutes (A) and 150 minutes (B) before 10^{-5} M acetylcholine (ACh) induced contractions in isolated longitudinal muscle-myenteric plexus preparation of rat jejunum. Effect of boiled IL1 β 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 1 β (IL1 β ; 10 ng/ml) added to the bath 90 minutes before acetylcholine (ACh) dose response curve (10^{-9} M to 10^{-3} 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 IL1 β 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

Figure 3: Inhibitory effect of interleukin 1 β (IL1 β) added to the bath 90 minutes before 10^{-5} 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.

induced contraction caused by IL1 β while TTX alone did not alter ACh induced jejunal contraction.

EFFECT OF IL1 β ON CONTRACTION OF DISPERSED JEJUNAL SMOOTH MUSCLE CELLS

Isolated cells of various lengths (range 26 to 83 μ m) were found in the unstimulated preparation. Exposure of smooth muscle cells to IL1 β (10 ng/ml) for 90 minutes had no significant effect on their length (52.7 (1.9) versus 55.6 (2.3) μ m). 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 IL1 β for 90 minutes, the contractile effect of

Figure 4: Effects of cycloheximide (Cyclo, 3.5×10^{-4} M), *N*^w-Nitro-L-Arginine (L-NNA, 3×10^{-4} M), tetrodotoxin (TTX, 10^{-6} M) on 10^{-5} M acetylcholine (ACh) induced contractile response after 90 minutes of incubation with interleukin 1 β (IL1 β , 10 ng/ml). Each column represents the mean (SEM) of values obtained from 12 strips. * $p < 0.05$ versus ACh alone.

ACh did not change significantly (40.3 (1.4) versus 42.3 (1.8) μ m).

Discussion

The results of this investigation show that IL1 β 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 IL1 β . The effect on jejunum was abolished by cycloheximide and TTX but persisted after pretreatment with L-NNA. The potent inhibitory motor effect of IL1 β seen in our experimental conditions was not caused by contamination by endotoxin (for example, lipopolysaccharide) because it was heat sensitive. The ability of IL1 β to inhibit ACh induced jejunal contraction corroborates previous reports showing that IL1 β inhibits the in vitro motility of gastric and other smooth muscles,¹⁰ including vascular and airway ones.^{16 17}

As inflammatory bowel disease occurs mainly in terminal ileum and right colon, we evaluated the effect of IL1 β on ACh ileal and proximal colonic contractile response. Our in vitro findings indicate that IL1 β (10 ng/ml), as seen for the jejunum, produced a similar inhibitory effect in rat ileum and colon. A recent in vivo study found that IL1 β affected the motility of various intestinal regions, suppressing postprandial motility at the jejunal site and modulating caecocolonic motor activity in conscious rats.⁹

As reported in previous studies performed on vascular and airway smooth muscle,^{16 17} the inhibitory effect of IL1 β on jejunal motility was found to be concentration dependent. The IL1 β concentrations that were effective in our study are similar to those that inhibited gastric motility¹⁰ and ACh release from rat longitudinal muscle preparation.⁸ This concentration range is similar to that measured in fresh intestinal mucosa of patients with IBD.¹⁸

Significantly more IL1 β 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- α ,² it is probable that IL1 β acts as a modulator of motility in both healthy and diseased intestine.

IL1 β 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 IL1 β on vascular and airway smooth muscle is time dependent.^{16 17} The delayed action of IL1 β suggests a mechanism involving the synthesis of a new molecule, which may well be a protein. As in the study of Main *et al*⁸ concerning the effect of IL1 β on ACh release, we found that cycloheximide blocked the IL1 β effect on intestinal motility completely, suggesting that a newly synthesised protein was involved.

Nitric oxide synthase could be the intermediate protein involved in the IL1 β effect. This enzyme has been found in vascular smooth muscle²⁰ and in myenteric plexus.²¹ 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 al*²² found that the IL1 β 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 affect the inhibitory action of IL1 β . Our findings are consistent with other findings in the rat in which nitric oxide synthase blockade did not change IL1 β induced relaxation of the proximal stomach.¹⁰ 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.^{23 24}

The fact that the contractile inhibitory effect of IL1 β was abrogated by prior administration of TTX strongly suggests the existence of a neural mediation pathway. Such mediation has also been suggested in IL1 β induced colonic hypersecretion in the rat.²⁸ Moreover, it has been shown that exogenous IL1 β inhibits both ACh⁸ and norepinephrine⁶ release from rat myenteric nerves. The notion that IL1 β receptors exist on the neurons of the myenteric plexus is indirectly supported by autoradiographic studies showing that IL1 β receptors occur on neurons of the central nervous system.²⁹ Other studies have shown that IL1 β can modulate invertebrate neuron functions,³⁰ 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 IL1 β 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 IL1 β has not been determined, IL1 β 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 IL1 β induces IL6 expression in rat intestinal smooth muscle cells.²⁷ Another peptide mediating IL1 β 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 IL1 β reflect changes in the acetylcholinesterase activity (enzyme which degrades ACh) and therefore in the bio-availability of ACh in presence of IL1 β . In fact, application of neostigmine, an acetylcholinesterase inhibitor, failed to affect the inhibitory action of IL1 β (data not shown). Our finding corroborates earlier findings indicating that IL1 β suppresses ACh release in LM-MP jejunal preparations superfused with Krebs' buffer solution containing physostigmine, an acetylcholinesterase inhibitor, suggesting that the IL1 β inhibitor effect could remain despite the inhibition of acetylcholinesterase.⁸ Moreover, the inhibitory effect of IL1 β was maintained even at high concentration. Finally, Palmer and Koch³² have recently shown that jejunal acute inflammation was associated with a decrease in acetylcholinesterase activity. Our study showed that IL1 β inhibits the contractile response of rat jejunal, ileal, 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 IL1 β 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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