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Effect of interleukin-1β on the ascending and descending reflex in rat small intestine

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Abstract

Acute inflammation of the intestine is associated with motility changes. We investigated the acute effect of inflammatory mediators such as interleukin-1β, interleukin-2 and tumor necrosis factor-α (TNF-α) on electrically stimulated ascending and descending reflex responses of the rat small intestine. Exogenous application of interleukin-1β caused a concentration-dependent inhibition of the oral contraction (0.1 ng/ml: $-22.9 \pm 3.8\%$, 10 ng/ml: $-57.0 \pm 7.4\%$, $P < 0.05$, $n = 10$) but had no effect on anal relaxation. The interleukin-1 receptor antagonist alone had no significant effect on the reflex response, but prevented the inhibitory effect of interleukin-1β (10 ng/ml: $-3.9 \pm 11.4\%$, $n = 8$). Interleukin-2 and TNF-α had no significant effect on the oral contractile and the anal inhibitory response (n.s., $n = 10$). Using reverse transcriptase polymerase chain reaction (RT-PCR) the presence of mRNA of the interleukin-1 receptor was demonstrated in the rat small intestine. Preincubation of the preparation with indomethacin (10^{-6} M), the histamine H_1 receptor antagonist, pyrilamine (10^{-8} M), and the histamine H_3 receptor antagonist, clobenpropit (10^{-8} M), decreased the oral contraction by $60.1 \pm 7.7\%$, $42.8 \pm 6.9\%$ and $44.4 \pm 14.2\%$ as well as the anal relaxation. These data suggest that acute administration of interleukin-1β inhibits the ascending and descending contractile reflex pathway and this effect seems not to be mediated by prostaglandins or histamine receptors.

Keywords: Interleukin; Prostaglandin; Histamine; Peristaltic reflex

1. Introduction

Acute inflammation of the gut is associated with profound changes in gastrointestinal motility and transit. The exact mechanisms of the motility changes are still unknown, but have been thought to involve inflammatory mediators released from immune-competent cells located in the mucosa and the gut wall (Cominelli et al., 1990; Hoang et al., 1994) as well as changes in transmitter release and nerve function in the myenteric plexus (Swain et al., 1991, 1992; Rühl et al., 1995; Valentine et al., 1996) and central modulation (Araujo et al., 1989; Tancredi et al., 1990). However, the motility changes could also be due to changes in the receptors or in the transduction pathways (Martinolle et al., 1997). Inflammatory mediators, particularly interleukin-1β, interleukin-2 and tumor necrosis factor α (TNF-α) have been suggested as possible mediators of inflammation-induced changes. Interleukin-1β is produced and released by various cell types, mainly activated monocytes and macrophages (Bautista et al., 1989) and its release is also stimulated by TNF-α. These cells are the most important source of TNF-α as well, which is known to share some characteristics with interleukin-1β. Interleukin-2, which is produced mainly by the Th_1-cell subtype, is involved in different immunological functions, especially T-cell activation, degranulation of mononuclear cells and histamine release (Nielsen et al., 1995). Interleukin-1β is present and released during acute inflammation of the intestine in animals (Cominelli et al., 1990) and humans (Youngman et al., 1993) and was postulated to be involved in the pathophysiological changes under these circumstances (Collins, 1996). Altered levels of interleukin-2 and TNF-α were reported in biopsies from inflamed intestine of patients with inflammatory bowel disease (Hoang et al., 1994). These cytokines have been suggested to release other inflammatory mediators such as prostaglandins or histamine (Nielsen et al., 1995) and have been suggested to alter the synthesis or release of peptides (Substance-P) (Hurst and Collins, 1993; Grider, 1995) and

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neurotransmitters (nitric oxide, acetylcholine, norepinephrine) in the enteric nervous system (Hurst et al., 1993; Main et al., 1993; Valentine et al., 1996). Therefore an immuno-neuronal interaction was postulated and the influence of cytokines, especially interleukin-1β, on neuronal transmission was investigated (Hori et al., 1988; Nakashima et al., 1989; Tancredi et al., 1990; Sawada et al., 1991; Li et al., 1992; Lin and Krier, 1995; Xia et al., 1995; Xia et al., 1996). Cytokine receptors were found in various neuronal tissues, in the brain (Katsuura et al., 1988; Araujo et al., 1989), and sympathetic ganglia (Hart et al., 1993), supporting a possible direct interaction with neuronal structures (Rada et al., 1991). Furthermore cultured neurons have been shown to synthesize and release interleukin-1β (Freidin et al., 1992). Preincubation of intestinal segments with interleukin-1β for 8 h increases the depolarization-induced release of substance P (Grider, 1995). However, little is known of the acute effect of cytokines on motility and especially on the ascending and descending reflex pathways.

Therefore, the aims of the present study were: (a) to investigate the acute effect of interleukin-1β, interleukin-2 and tumor necrosis factor-α on reflex activity in the rat small intestine with no inflammation, (b) to study the possible physiological role of endogenous interleukin-1β, using a specific receptor antagonist, (c) to study the presence of interleukin-1β receptors in the lamina propria containing the myenteric plexus, (d) to investigate other mediators such as histamine (Leurs et al., 1991; Bertaccini and Coruzzi, 1995) and prostaglandins (Theodorou et al., 1994; Rühl et al., 1995), which could be involved in the mediation of the interleukin-1β effect.

2. Materials and methods

Male Wistar rats (300–400 g) were killed with sodium pentobarbital intraperitoneally (80 mg/kg). The ileum was immediately removed and kept in oxygenated Krebs–Ringer buffer (KRB). A modified reflex model to record ascending and descending reflex activity was used (Holzer, 1989; Allescher et al., 1992b). A segment of ileum (10 cm in length) was carefully dissected and the mesenteric arcade was removed. The gut segment was placed in a 35-ml organ bath filled with KRB, gassed with 95% O_2, 5% CO_2 and maintained at 37°C by a thermostatic water bath. A glass rod 2 mm in diameter was passed through the lumen and fixed to the lower part of the gut. Mechanical activity of the circular muscle was recorded with force-displacement transducers attached by thin threads to the serosa 2 cm aborally and orally of the platinum stimulation electrode. The oral and anal ends of the ileum were tied onto polyvinyl tubing (outer diameter 4 mm), maintaining the natural length of the gut segment. Two flat platinum electrodes (0.5 cm × 1.0 cm), which were 1 cm apart were inserted between the two recording sites (Allescher et al., 1992b). Then the preparation was allowed to equilibrate for at least 30 min. Contractile changes were recorded using Grass FTO3C force transducers and a Sensormedics R611 chart recorder. Field stimulation impulses for neural responses were applied from a Grass S11 stimulator (Grass, Quincy, MA, USA), stimulating for 15 s at intervals of 2 min with standard parameters of 20 V pulse strength, 3 pps pulse frequency, 2 ms pulse width. The stimulus signals were recorded simultaneously with the motility recording on the chart recorder using an A/C coupler (Beckman). After recording the reflex response, we determined the area under the curve with a magnetic scan system (Sigma Scan, Jandel Scientific, USA).

2.1. Experimental design

The gut segment was stimulated every 2 min and this periodic stimulation was maintained throughout the experiment. After an equilibration period of 30 min, a stable response to the electrical stimulation was established (identical contractions in response to at least 4 consecutive stimuli), which were used as the control response. Drugs were added 60 s after the last stimulation and before the next electrical stimulation. The substances were added to the bath in volumes of 35 μl. At least four reflex responses were elicited for each substance concentration before the next concentration was applied. Appropriate control experiments were performed with the vehicles over a period of 3 h to exclude non-specific effects. At the end of the experimental protocol the buffer (KRB) was exchanged several times and after a period of 15 min a control recording was performed after washout.

2.2. Interpretation of the motility response

Electrical field stimulation caused an ascending contraction and a descending relaxation which was followed by a contractile response. We assumed that the contractile response occurred as an event propagated from the oral contraction. Alternatively, the anal contraction could occur as an 'off-response' to the stimulation. However, the anal contraction, like the oral contraction, is dependent on ganglionic transmission as it is blocked by hexamethonium.

2.3. RNA isolation and polymerase chain reaction (PCR) amplification

For RNA preparation, the two muscle layers were separated under a stereomicroscope. The gut segment was passed onto a glass pipette and after an incision of the longitudinal muscle layer with a scalpel, the longitudinal muscle layer with adherent myenteric plexus was removed with a cotton swab. Then the circular muscular layer was scraped off using a scalpel. Tissues obtained from the longitudinal muscle/myenteric plexus layer and the circu-

lar muscle layer were placed in lysis solution (mercaptoethanol and guanidinisothiocynate) and were subsequently homogenized with a Polytron PT20 homogenizer at a setting of approximately 1500 rpm for 20 s (4 × 5 s) on ice. Portions, 500 μl, of the homogenate were then used to prepare RNA, using the commercially available 'micro RNA isolation kit' (Stratagene, Heidelberg, Germany), which is based on chloroform and phenol extraction. The total yield from 0.5 g longitudinal and circular muscle layers was 57 μg, and 173 μg RNA, respectively. Using this RNA yield, 1 μg was used for DNase treatment (15 min, 1 IU DNaseI, room temperature). RNA was reverse transcribed to complementary DNA, using AMV reverse transcriptase and oligo-$(dT)_{15}$ primer (20 μM) (both from Boehringer Mannheim, Germany) at 25°C over 10 min and 42°C over 60 min. At this point, the newly synthesized cDNA was stored at −20°C until PCR experiments were performed. PCR amplification of the interleukin-1 receptor was carried out using primers specific for the known interleukin-1 receptor subtype I sequence in rats (Hart et al., 1993; Prinz et al., 1997). Primers were selected by software analysis (DNAstar, UK) using the receptor sequences available on GeneWorks® (Intelligenetics, San Francisco, CA, USA). Primers for the interleukin-1 subtype I receptor were: 5′-CTT GCC GCA CGT CCT ACA ATA CC-3′ for the sense primer and 5′-CGG GGA AGA AAA TCA GAG CAG GAG-3′ for the antisense primer. The calculated PCR product length was 547 base pairs for interleukin-1 receptor amplification. Annealing temperature was 61°C (60 s), and PCR amplification was carried out over 35 cycles using Primezyme-Polymerase (Biometra, Göttingen, Germany). PCR products were separated by agarose gel electrophoresis.The bands were cut out, DNA was extracted using the Gel Extraction Kit (Quiagen, Hilden, Germany), and then sequenced by a commercial institute (Medigene, Martinsried, Germany) (Hart et al., 1993; Prinz et al., 1997).

2.4. Contractility studies with isolated smooth muscle strips

The experimental model and protocol for investigation of the inhibitory response were as previously described (Allescher et al., 1992a). Six segments of full thickness strips were prepared from the terminal ileum (length 1.5–2 cm), and fixed to a hook on a holder. Holders with tissue were placed in a jacketed organ bath containing 3 ml Krebs–Ringer bicarbonate solution gassed with 95% O_2, 5% CO_2 and maintained at 37°C by circulating water through the jackets. The free end of one segment was connected with a thread to an isometric force transducer (Swegma force displacement transducer SG 4-500, Swegma Sweden); 1 g tension was applied to the muscle, and the preparation was allowed to equilibrate for at least 30 min. Changes in tension were amplified by Hellige couplers and recorded on a Rikadenki chart recorder. The segments were precontracted by addition of the muscarinic receptor agonist, carbachol (10^{-6} M) and the effect of interleukin-1β (10 ng/ml) was studied on the contractile plateau.

2.5. Statistics

The data are given as means ± standard error (means ± S.E.M.) expressed as percent of control. The mean response to four subsequent stimulations before addition of a substance or drug were used as control values. *n* indicates the number of independent observations with different

Table 1
The effect of different interleukins and TNF-α on the oral and anal excitatory and inhibitory reflex response in rat small intestine, as area under the curve (mean ± S.E.M.)

	n	Oral contraction	Anal relaxation	Anal contractile
Interleukin-1β (human, recombinant)				
0.1 ng/ml	12	−22.9 ± 3.8%	+1.5 ± 4.4% n.s.	−35.6 ± 6.3%[a]
1.0 ng/ml	12	−38.5 ± 7.5%[b]	+6.3 ± 15.8% n.s.	−59.1 ± 5.2%[b]
10 ng/ml	12	−57.0 ± 7.4%[c]	−6.9 ± 6.8% n.s.	−76.7 ± 3.8%[c]
Interleukin-1β (mouse, recombinant)				
0.1 ng/ml	3	−19.4 ± 9.6%[a]	−8.7 ± 14.6% n.s.	−39.6 ± 2.1%[a]
1.0 ng/ml	3	−32.8 ± 8.2%[b]	+1.2 ± 6.4% n.s.	−56.3 ± 7.8%[a]
10 ng/ml	3	−46.3 ± 8.5%[c]	−3.0 ± 3.7% n.s.	−72.0 ± 11.8%[b]
Interleukin-2				
0.1 ng/ml	10	−14.2 ± 10.8% n.s.	−10.9 ± 10.8% n.s.	−30.7 ± 12.3%[a]
1.0 ng/ml	10	−25.9 ± 14.0% n.s.	−13.3 ± 11.0% n.s.	−52.0 ± 9.5%[b]
10 ng/ml	10	−24.0 ± 12.6% n.s.	−13.0 ± 12.4% n.s.	−77.6 ± 4.8%[c]
TNF-α				
0.1 ng/ml	10	−1.7 ± 1.9% n.s.	−0.9 ± 5.3% n.s.	−1.2 ± 3.8% n.s.
1.0 ng/ml	10	−2.9 ± 2.5% n.s.	−0.5 ± 14.1% n.s.	−4.3 ± 6.1% n.s.
10 ng/ml	10	−7.7 ± 3.5% n.s.	+3.9 ± 6.3% n.s.	−11.2 ± 5.7% n.s.

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

segments of small intestine. Analysis of variance (ANOVA) with Bonferroni correction for multiple comparison was used to compare the mean values and a probability of less than 0.05 was considered significant.

2.6. Drugs

The drugs used were atropine, carbachol, hexamethonium, tetrodotoxin, indomethacin, mercaptoethanol (Sigma, München, Germany), interleukin-1β (recombinant human): Bachem-Feinchemikalien, Germany, interleukin-1β (recombinant mouse): Genzyme Diagnostics, Cambridge, interleukin-1 receptor antagonist (recombinant human) (R&D Systems Europe), AMV reverse transcriptase, oligo-$p(dT)_{15}$ primer (Boehringer-Mannheim, Germany), micro RNA isolation kit (Stratagene, Heidelberg Germany), interleukin-1β primer (Medigene, Martinsried, Germany), guanidinisothiocynate (Live Technology, Eggenstein, Germany), N^G-nitro-L-arginine-methyl-ester (L-NAME), interleukin-2, TNF-α (Bachem Feinchemikalien, Heidelberg, Germany), clobenpropit (Tocris Cookson, Bristol), pyrilamine maleate, ranitidine hydrochloride (Research Biochemicals, Biotrend, Köln, Germany).

The drugs were dissolved in purified distilled water, stored at −20°C, freshly thawed 10 min before use and further diluted in KRB. Only indomethacin was dissolved in trizma base and NaCl (0.2 M, pH = 10). The drugs were added in 35-μl portions.

3. Results

3.1. Characterization of the experimental system

Electrical stimulation of the gut segment between the recording sites caused an oral contraction, and an anal relaxation which was followed by a contractile response. All motility responses caused by electrical field stimulation were abolished by tetrodotoxin (3×10^{-7} M: oral contraction: $-96.1 \pm 4.5\%$, anal relaxation: $-93.6 \pm 4.5\%$, anal contractile response: $-100 \pm 0.0\%$, $n = 5$) as well as hexamethonium (10^{-4} M: oral contraction: $-100 \pm 0.0\%$, anal relaxation: $-90.17 \pm 4.7\%$, anal contractile response: $-100 \pm 0.0\%$, $n = 5$) demonstrating neuronal and ganglionic transmission, which is consistent with the results reported (Holzer, 1989; Allescher et al., 1992b). Blockade of muscarinic receptors with atropine (10^{-6} M)

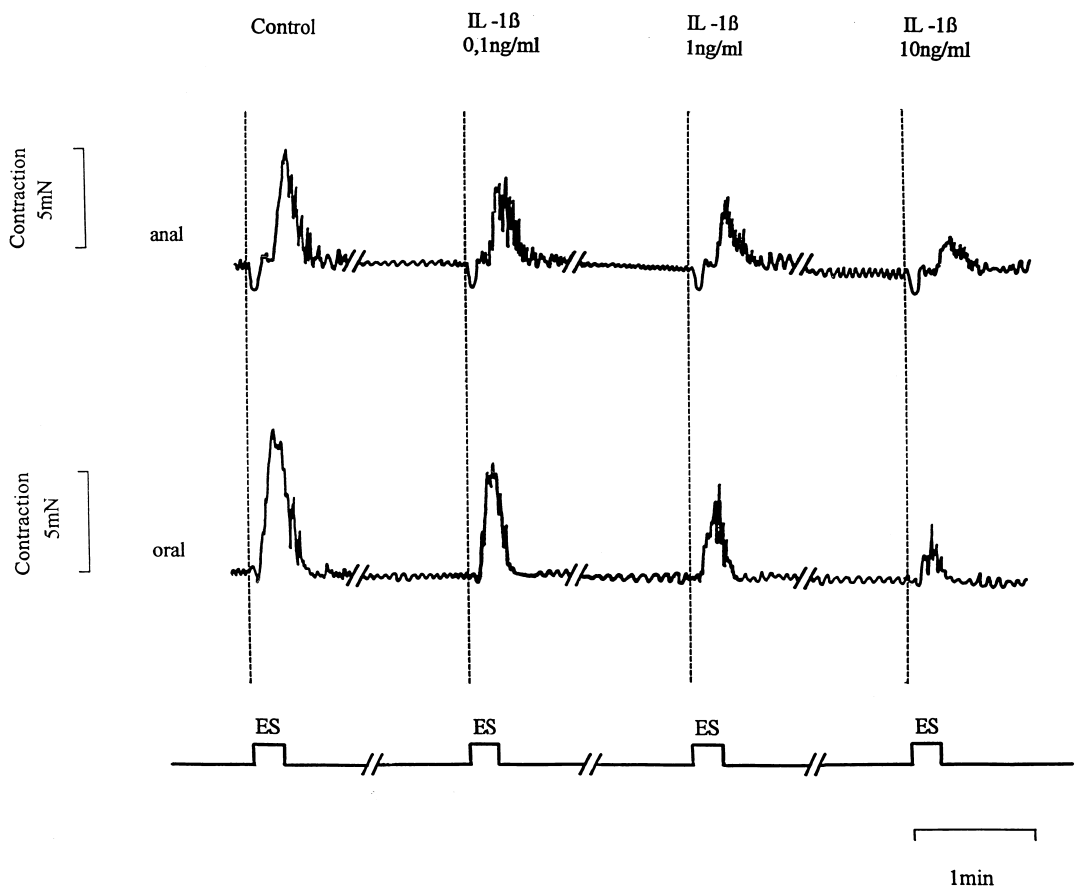

Fig. 1. Typical tracing of the inhibitory effect of exogenous human recombinant interleukin-1β (IL-1β: 0.1 ng/ml; 1 ng/ml; 10 ng/ml) on the ascending contraction (lower panel) and the descending contraction (upper panel) induced by electrical stimulation (ES). The descending relaxation was not affected (upper panel).

decreased the oral and anal contraction (atropine 10^{-6} M: oral: $-93.8 \pm 4.7\%$, $n = 8$, anal: $-86.4 \pm 10.9\%$, $n = 5$), whereas the anal relaxation was significantly increased by cholinergic blockade (10^{-6} M: $+221.2 \pm 82.4\%$, $n = 6$). The anal relaxation was concentration dependently blocked by the NO synthesis inhibitor, L-NAME (10^{-5} M: $-20 \pm 6.9\%$, 10^{-4} M: $-65 \pm 9.8\%$, 3×10^{-4} M: $-93 \pm 4.5\%$, $n = 10$).

3.2. Effect of interleukin-1β

Exogenous acute application of interleukin-1β (human recombinant) caused a concentration-dependent inhibition of the oral contraction and the anal contractile response but had no effect on the anal relaxation. Mouse recombinant interleukin-1β had a similar inhibitory effect on the oral and anal contraction, and did not alter anal relaxation either (Table 1, Fig. 1). This inhibitory effect could be reversed by washout. When interleukin-1β was not washed out the inhibitory effect of interleukin-1β lasted up to 2 h.

To test the effect of endogenous interleukin-1, the interleukin-1 receptor antagonist (10 ng/ml) was tested on the reflex response. When given alone, interleukin-1 receptor antagonist showed no significant effect on either oral or anal contraction (10 ng/ml: $+11.9 \pm 2.5\%$, n.s., $n = 8$).

However, when the preparation was pretreated with interleukin-1 receptor antagonist (10 ng/ml) (8 min before), it blocked the inhibitory action of interleukin-1β (human) on the oral and anal contraction (interleukin-1β + interleukin-1 receptor antagonist: 1.0 ng/ml: $+6.0 \pm 8.6\%$, 10 ng/ml: $-3.9 \pm 11.4\%$, $n = 8$) (Fig. 2).

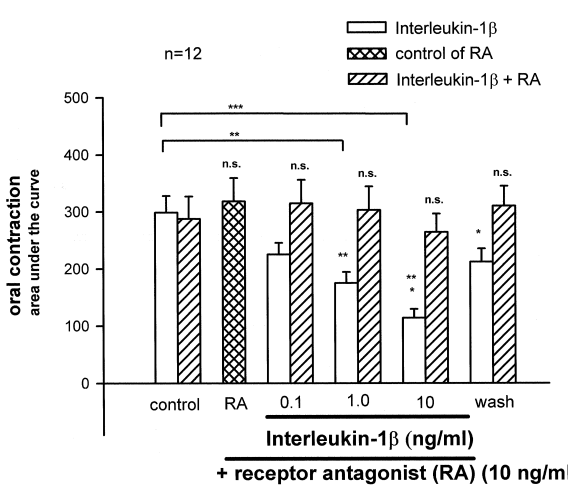

Fig. 2. Interleukin-1β (0.1 ng/ml; 1 ng/ml; 10 ng/ml) alone (open bars) caused a significant inhibition of the ascending contraction. This inhibitory effect of interleukin-1β was prevented by pretreatment with the interleukin-1 receptor antagonist (RA, 10 ng/ml) (hatched bars) which had no effect when given alone (cross-hatched bar). Mean ± S.E.M., $n = 12$, $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$.

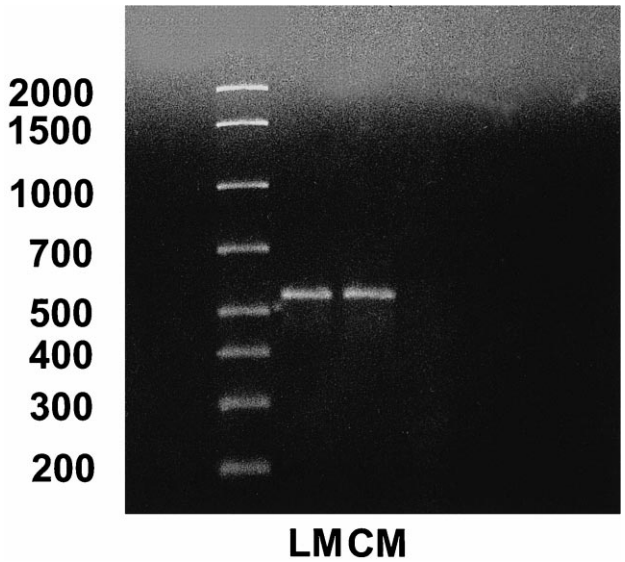

Fig. 3. The reverse transcriptase polymerase chain reaction with primers specific for the interleukin-1 subtype I receptors in longitudinal muscle myenteric plexus (LM) and circular muscle (CM) in rat small intestine. Agarose gel electrophoresis showed a single specific band with 547 base pairs in the LM and CM preparations.

3.3. RT-PCR of the interleukin-1 receptor in nerve muscle preparation of longitudinal muscle and circular muscle of rat small intestine

To investigate whether the interleukin-1 receptor could be found in the nerve-muscle layer of the rat small intestine, mRNA was extracted from the muscle layer (longitudinal muscle, circular muscle) with the attached intestinal plexi. RT-PCR with specific primers for rat interleukin-1 subtype I receptor was performed. The RT-PCR in the LM and CM fraction demonstrated a single band with approximately 547 base pairs, which is consistent with the expected size of the PCR product. To verify the identity of the product amplified by PCR, the band was cut out and sequenced. Analysis of the amino-acid sequence of the RT-PCR product showed 99% homology to that of the known rat interleukin-1 subtype I receptor, demonstrating that the identified mRNA sequence is encoding this receptor (Fig. 3).

3.4. Effect of interleukin-1β on isolated smooth muscle strips

In order to investigate whether interleukin-1β would elicit a direct relaxant effect on the smooth muscle, the acute effect of interleukin-1β (10 ng/ml) on pre-contracted isolated muscle strips was studied. Carbachol (10^{-6} M) caused an instantaneous contraction of the isolated smooth muscle (1.51 ± 0.23 mN, $n = 7$) which was not significantly influenced by addition of interleukin-1β (10 ng/ml) (1.37 ± 0.19 mN, $n = 7$, n.s.).

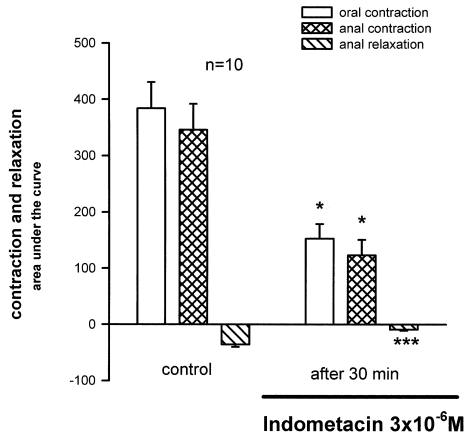

Fig. 4. The inhibitory effect of indomethacin at a concentration of 3×10^{-6} M, after an incubation time of 30 min on the ascending contraction (open bars), the descending contractile (cross-hatched bars) and descending relaxant (hatched bars) response in rat small intestine. Mean ± S.E.M., $n=10$, $*\ P<0.05$ and $***\ P<0.001$ versus control.

3.5. Effect of interleukin-2

Interleukin-2 had no significant effect on the anal relaxatory response. But, in contrast to interleukin-1β, interleukin-2 caused only a significant inhibition of the contraction occurring on the anal side following the relaxant response, whereas the effect on the oral contractile response did not reach significance (Table 1).

3.6. Effect of tumor necrosis factor α

Exogenously applied TNF-α at a concentration of 0.1 ng/ml, 1.0 ng/ml and 10 ng/ml had no significant acute effect on either ascending or descending relaxant or contractile reflex responses (Table 1).

3.7. Blockade of cyclooygenase with indomethacin

Pre-incubation of the reflex preparation with the cyclooxygenase inhibitor, indomethacin (3×10^{-6} M) alone, decreased the oral ($-60.1\pm7.7\%$, $P<0.05$, $n=10$) and anal ($-65.6\pm6.7\%$, $P<0.05$, $n=8$) contractile as well as the anal relaxant response ($-73.6\pm6.5\%$, $P<0.05$, $n=10$) (Fig. 4). After indomethacin the response varied and did not allow systematic testing of interleukin-1β.

3.8. Blockade of histamine H_1, H_2 and H_3-receptors

The histamine H_1-receptor blocker, pyrilamine maleate, significantly decreased the ascending and descending contractile response in a concentration-dependent manner (ascending contraction: 10^{-10} M: $-31.9\pm8.6\%$, 10^{-8} M: $-42.8\pm6.9\%$, 10^{-6} M: $-66.2\pm7.6\%$, descending contraction: 10^{-10} M: $-37.5\pm9.5\%$, 10^{-8} M: $-56.7\pm8.8\%$, 10^{-6} M: $-66.0\pm10.3\%$, $P<0.05$, $n=11$). A similar effect was obtained with the selective histamine H_3-receptor blocker, clobenpropit (ascending contraction: 10^{-10} M: $-22.6\pm9.4\%$, 10^{-8} M: $-44.4\pm14.2\%$, 10^{-6} M: $-78.2\pm3.7\%$, descending contraction: 10^{-10} M: $-28.0\pm6.8\%$, 10^{-8} M: $-57.3\pm12.4\%$, 10^{-6} M: $-88.1\pm7.2\%$, $P<0.05$, $n=8$). Neither pyrilamine nor clobenpropit had any significant effect on anal relaxation (n.s.). The selective histamine H_2-receptor blocker, ranitidine, had no significant effect on ascending or descending responses (n.s., 10^{-7} M, 10^{-8} M) (Fig. 5).

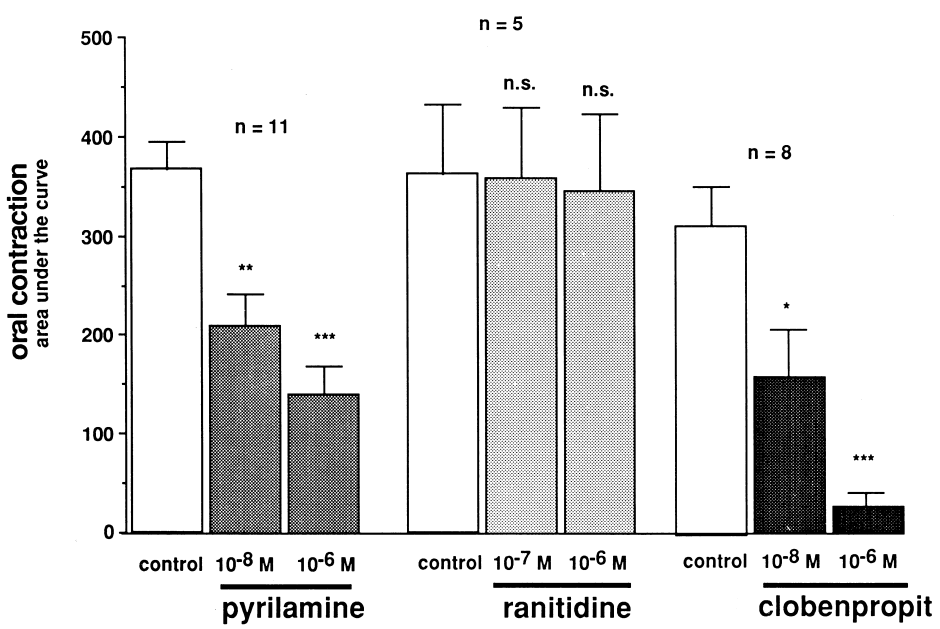

Fig. 5. The effect of different histamine receptor antagonists on the ascending oral contraction. The H_1-antagonist, pyrilamine (10^{-8} M, 10^{-6} M), and H_3-antagonist, clobenpropit (10^{-8} M, 10^{-6} M), caused a significant inhibition of the oral contraction whereas the H_2-antagonist, ranitidine (10^{-7}, 10^{-6} M), had no effect (n.s.). The open bars give the control response before the administration of the respective antagonist and the filled bars give the contractile effects after application of pyrilamine, ranitidine and clobenpropit as indicated. Mean ± S.E.M, $*\ P<0.05$, $**\ P<0.01$, $***\ P<0.001$.

4. Discussion

The aim of the present study was to elucidate the acute effects of different cytokines, especially interleukin-1β on the reflex pathway in rat intestine. The results demonstrated that acute administration of interleukin-1β causes suppression of the ascending reflex of an uninflamed gut segment, but does not alter the descending relaxation. The acute inhibitory effect of interleukin-1β was concentration-dependent and specifically antagonized by an interleukin-1 receptor antagonist. As shown in other experimental systems, interleukin-1 receptor antagonist blocked the interleukin-1β effect in a concentration range around 10 ng/ml (Hannum et al., 1990). Interleukin-1 receptor antagonist itself had no direct effect on the ascending and descending contractile and relaxatory response when given alone. As interleukin-1 receptor antagonist, which would block effects from endogenous interleukin-1β, had no effect on the ascending and descending contractile and relaxant reflex pathway, we conclude that endogenous interleukin-1β has no acute effect on the reflex pathway in the absence of inflammation.

Earlier neurophysiological studies on aplysia neurons had pointed to a possible site of action of interleukin-1β on neuronal tissue (Sawada et al., 1991). Interleukin-1β and its receptor could be located in the central nervous system (CNS) of rats (Katsuura et al., 1988) and neuronal transmission altered by interleukin-1β was shown in different neurons in the CNS (Hori et al., 1988; Nakashima et al., 1989; Li et al., 1992) and pelvic plexus (Lin and Krier, 1995). Xia et al. (1995, 1996) demonstrated that, in the submucous plexus of guinea pig intestine, interleukin-1β suppressed the amplitude of electrically evoked inhibitory post synaptic potentials (IPSPs). They postulated a presynaptic mechanism, as noradrenergic IPSPs and nicotinergic excitatory post synaptic potentials (EPSPs) could be suppressed by interleukin-1β, whereas interleukin-1β could not alter the effect of norepinephrine or acetylcholine at their postsynaptic receptors. These results indicated an acute direct action of interleukin-1β, pointing to a possible mechanism of the acute inhibitory effect of interleukin-1β on the reflex pathway in the enteric nervous system.

To determine whether possible binding sites for IL-1β are present in the rat small intestine, interleukin-1 subtype I receptor mRNA expression was investigated with RT-PCR in the nerve-muscle layer of the small intestine. The single PCR product opained showed the expected size of the interleukin-1 receptor and sequence analysis confirmed the identity of the RT-PCR product as part of the known sequenced interleukin-1 receptor of the rat. These data demonstrated that mRNA for the interleukin-1 subtype I receptor is expressed in the rat small intestine. The RT-PCR experiments cannot differentiate the localization of the mRNA expression, thus could not answer the question as to whether the expression occurs in muscle cells, neurons or another cell type. To investigate a possible direct inhibitory effect of interleukin-1β on smooth muscle, contractile studies with isolated muscle strips were performed. No acute effect of interleukin-1β (10 ng/ml) on pre-contracted muscle strips could be found in this preparation. This negative finding is consistent with the results of Aube et al. (1996) who showed that, in isolated smooth muscle strips of the rat intestine, basal contractility was not affected by interleukin-1β, and acetylcholine-induced contractions were only inhibited after 90 or 150 min.

The fact that we could not find an acute effect of interleukin-1β on the descending inhibitory reflex, which can be blocked by nitric oxide synthase inhibitors demonstrates that the effect appears to be selective for the excitatory pathway. Together with the negative finding with isolated muscle strips, this would also be consistent with a neuronal effect. However, based on our results, we cannot determine the exact site of action of interleukin-1β. But the reported acute changes of neuronal function (Hori et al., 1988; Nakashima et al., 1989; Sawada et al., 1991; Li et al., 1992; Lin and Krier, 1995; Xia et al., 1995, 1996) further support a neuronal site of action.

Another possible but less likely mechanism could be via the release of oxygen free radicals. Interleukin-1β and interleukin-2 are known to release oxygen free radicals from macrophages and monocytes and recently the interleukin-2-induced release of oxygen free radicals by neutrophils was shown (Klausner et al., 1991). According to our data it might be possible that interleukin-1β and/or interleukin-2 act via the release of free radicals. In contrast to interleukin-1β, interleukin-2 does not alter the ascending contractile and descending relaxant reflex response but inhibits the anal contractile response following relaxation. This result itself is surprising, and based on our data we cannot give an exact reason for this selective action. It could indicate that oral and anal contraction might be elicited, inhibited or mediated differently. There is evidence for a direct effect of interleukin-2 on neuronal tissue in the CNS (Araujo et al., 1989; Tancredi et al., 1990), which might also point to a possible neuronal mechanism for interleukin-2 action.

TNF-α was shown to release interleukin-1β from isolated macrophages after an incubation time of 24–48 h (Dinarello et al., 1986). We examined the acute effect of TNF-α on the reflex pathway at a concentration of 10 ng/ml, a concentration, which was also used by others (Hurst and Collins, 1994). In our experiments, TNF-α did not influence the reflex pathway, indicating that acute exogenous application of TNF-α under our experimental conditions has no, or only a minor effect on interleukin-1β release.

Interleukin-1β has been reported to act via, or to interact with, the arachidonic acid system and prostaglandins have been suggested to be involved in various interleukin-1β-mediated effects (Theodorou et al., 1994; Rühl et al., 1995). Furthermore interleukin-1β can alter histamine release, and histamine was shown to change electrical be-

haviour in myenteric neurons in guinea pig small intestine and thus it is speculated that histamine receptors are involved (Nemeth et al., 1984). Therefore these two possible pathways were investigated. Indomethacin, a blocker of prostaglandin synthesis, and various histamine receptor antagonists (histamine H_1 receptor antagonist pyrilamine, histamine H_2 receptor antagonist ranitidine and histamine H_3 receptor antagonist clobenpropit) were used. Interleukin-1 has been shown to increase prostaglandin synthesis in several systems (Theodorou et al., 1994; Rühl et al., 1995) and these effects were antagonized by blockers of cyclooxygenase. In the intestine however, the induction of prostaglandins by interleukin-1β was demonstrated only as a long-term effect (Rühl et al., 1995). Blocking both the arachidonic acid and the histamine pathway also decreased the responses, similarly to the acute effect of interleukin-1β. Therefore it was not possible to test whether these blockers would prevent the inhibitory effect of interleukin-1β. However, concluding from the effect observed, it seems unlikely that the acute interleukin-1β effect is mediated by the release of histamine or by an augmentation of prostaglandin synthesis. Our data support the possibility that a possible slow, permanent basal release of histamine as well as prostaglandins in our experimental system might be involved in the responsiveness to the stimuli for reflex contraction.

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