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Interleukin-1 receptor antagonist displays intrinsic agonist activity on rat gastric fundus motility in vitro

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Abstract

It has been shown previously that both forms of interleukin-1, 1α and 1β, produce dose-dependent relaxation of the rat gastric fundus in vitro, accompanied by an increased production and release of eicosanoids. This effect appears to be mediated, at least in part, by leukotrienes, since the inhibition of 5-lipoxygenase by specific drugs counteracts interleukin-1-induced gastric relaxation. In the present study, we attempted to antagonize interleukin-1-induced inhibition of gastric fundus motility with a interleukin-1 receptor antagonist. Surprisingly, the interleukin-1 receptor antagonist itself possessed interleukin-1-like agonist activity, since: (a) it produced rapid, dose-dependent relaxation of the rat gastric fundus, with an estimated EC_{50} of 70 pg/ml and a maximal effect at 10 ng/ml; (b) interleukin-1 receptor antagonist-induced relaxation was dose dependently inhibited by *N*-(3-phenoxycinnamyl)acetohydroxamic acid (BW A4c), a specific inhibitor of 5-lipoxygenase; (c) in the first 5 min after its addition to the bath solution, interleukin-1 receptor antagonist produced a significant increase in prostaglandin E_2 release from the gastric strips. This evidence suggests that, shortly after receptor occupancy, in this experimental model interleukin-1 and interleukin-1 receptor antagonist share the same pattern of mechanical and biochemical activities.

Keywords: Interleukin-1 receptor antagonist; Interleukin-1; Prostaglandin E_2; 5-Lipoxygenase; Stomach, rat

1. Introduction

The interleukin-1 receptor antagonist has been characterized as a polypeptide of approximately 17 kDa, which is produced by the same cells that synthesize interleukin-1 (Eisenberg et al., 1990; Hannum et al., 1990). Interleukin-1 receptor antagonist shows an amino acid homology of 26% and 19% with interleukin-1β and interleukin-1α, respectively (Dinarello, 1991).

We have previously shown that both interleukin-1α and tumor necrosis factor are capable of inducing dose-dependent relaxation in rat gastric fundus strips in vitro (Montuschi et al., 1993). Similar results have been obtained with interleukin-1β (Montuschi et al., 1994). In the present study, we attempted to antagonize interleukin-1-induced inhibition of gastric fundus motility with interleukin-1 receptor antagonist. Interestingly, interleukin-1 receptor antagonist itself produced rapid, dose-dependent relaxation of the rat gastric fundus. Further experiments were conducted to investigate whether, in this experimental model, interleukin-1 receptor antagonist and interleukin-1 share the same pattern of mechanical and biochemical actions.

2. Materials and methods

2.1. Experimental procedures

Experimental procedures have been described in detail elsewhere (Montuschi et al., 1994). Briefly, longitudinal smooth muscle strips (3 × 20 mm) were prepared from gastric fundi of male and female Wistar rats. Strips were placed in 5-ml organ baths, gassed with 95% O_2-5% CO_2 and incubated at 37°C in

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standard Krebs solution, supplemented with 5-hydroxytryptamine (5-HT) 3 μM (to increase the basal tone) unless otherwise stated. Changes in strip length were magnified 5–10 times and recorded by means of Harvard auxotonic transducers coupled with Rikadenki R-01 single-pen recorders.

The tissues were allowed to equilibrate for 1 h during which the bath solution was changed every 10 min. After the equilibration period, the bath solution was renewed every 5 min between cytokine applications. At the end of each experiment a control maximal relaxation was produced in each strip with 100 μM papaverine. Cytokine-induced relaxation was considered maximal if it was not significantly different from the control relaxation.

Experiments with electrical field stimulation were conducted in non-adrenergic non-cholinergic conditions, as described elsewhere (D'Amato et al., 1992). Electrical field stimulations ranged from 1 to 16 Hz, with a 1-min duration.

2.2. Release experiments

In some experiments, 1-ml aliquots of bath solution were taken before the addition of interleukin-1 receptor antagonist and during relaxations induced by the cytokine (10–1000 pg/ml), or control relaxing agents, for simultaneous determination of prostaglandin E_2 and prostaglandin $F_{2\alpha}$. Prostaglandins released by the tissue were estimated in the medium by radioimmunoassay techniques, as previously described (Navarra et al., 1992).

2.3. Cytokines

Interleukin-1β, kindly provided by Dr. S. Poole of the National Institute for Biological Standards and Control (South Mimms, Potter Bar, Hertfordshire, UK), was a freeze-dried preparation of the recDNA human type (batch 86/680). Each ampoule contained 1 μg of interleukin-1β, with an assigned potency of 100 000 IU. The cytokine was resuspended in 10 ml phosphate buffer 0.05 M containing 0.2% human serum albumin (Sigma Chemical Co., St. Louis, MO, USA), and aliquoted into 10-ng fractions. Recombinant human interleukin-1α was obtained from Amersham (Little Chalfont, Buckinghamshire, UK). Interleukin-1α was resuspended and aliquoted in the same manner as interleukin-1β.

Recombinant human interleukin-1 receptor antagonist was obtained from British Biotechnology Products (Abington, Oxon, UK). Each ampoule contained 10 μg of a freeze-dried preparation with a specific activity of 10 000 IU/mg and an endotoxin content of less than 1 pg/μg of cytokine. An alternative source of interleukin-1 receptor antagonist, Bachem Feinchemikalien (Bubendorf, Switzerland), was also tested in order to confirm the reproducibility of the results. Interleukin-1 receptor antagonist was resuspended in the same man-

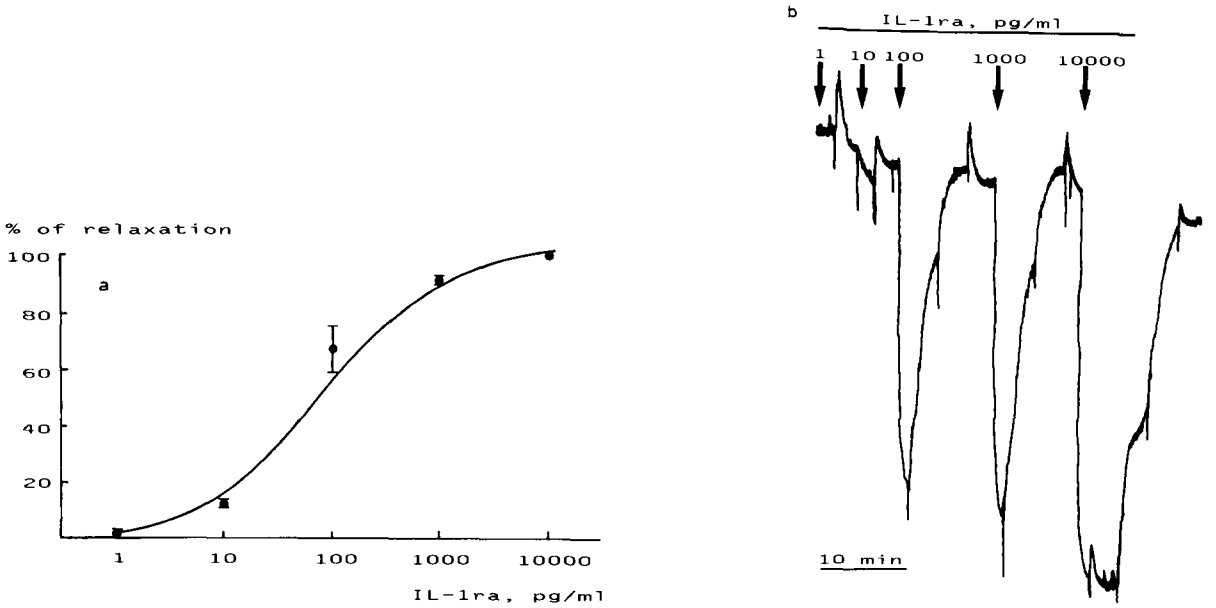

Fig. 1. The relaxant effect of interleukin-1 receptor antagonist on rat gastric fundus in vitro. (a) Log dose-response curve. The data are expressed as mean (±S.E.M., $n = 8$) percentages of maximal relaxation, obtained for each strip with 100 μM papaverine. (b) Representative tracing from 8 experiments with interleukin-1 receptor antagonist. Arrows: addition of interleukin-1 receptor antagonist to the incubation medium. Washout at the plateau of the response (3 min after the addition of the cytokine).

ner as the other cytokines, and aliquoted into 0.5-μg fractions.

On the day of the experiment, aliquots of interleukin-1β, interleukin-1α and interleukin-1 receptor antagonist were diluted to the desired concentrations in Krebs solution and added to the organ baths in 50-μl volumes.

2.4. Drugs

N-(3-Phenoxycinnamyl)acetohydroxamic acid (BW A4c) was kindly supplied by Dr. L.G. Garland (Wellcome Research Laboratories, Beckenham, Kent, UK).

Other drugs used were: 5-HT creatinine sulfate, noradrenaline HCl, papaverine HCl and sodium nitroprusside (Sigma).

BW A4c was dissolved in absolute ethanol and diluted 1/100 in Krebs solution. Noradrenaline was dissolved in 5% ascorbic acid and diluted in Krebs solution. All other drugs were dissolved in distilled water and diluted to the desired concentration in Krebs solution.

2.5. Statistical analysis

All results are given as means ± S.E.M. Data from release experiments were analyzed by two-way analysis of variance (ANOVA), for the response variability due to either treatments or different strips of tissue, and subsequent Student's *t*-test for comparisons among group means. Differences were taken as significant if $P < 0.05$.

3. Results

3.1. Functional experiments

This study originally aimed at antagonizing the interleukin-1-induced gastric relaxation with interleukin-1 receptor antagonist. In preliminary experiments, the direct effect of the latter protein on the gastric strips was investigated. Surprisingly, interleukin-1 receptor antagonist itself caused a rapid, dose-dependent relaxation of the strips which was similar to, though less potent than, that produced by interleukin-1α and interleukin-1β. The estimated EC_{50} was 70 pg/ml and maximal relaxation was obtained at 10 ng/ml (Fig. 1a and b). Interleukin-1 receptor antagonist purchased from Bachem Feinchemikalien, used as a control to test the reproducibility of these results, was slightly less potent than the British Biotechnology protein, as it displayed an estimated EC_{50} of 200 pg/ml and maximal relaxation at 10 ng/ml; all the subsequent experiments were conducted with interleukin-1 receptor antagonist obtained from British Biotechnology.

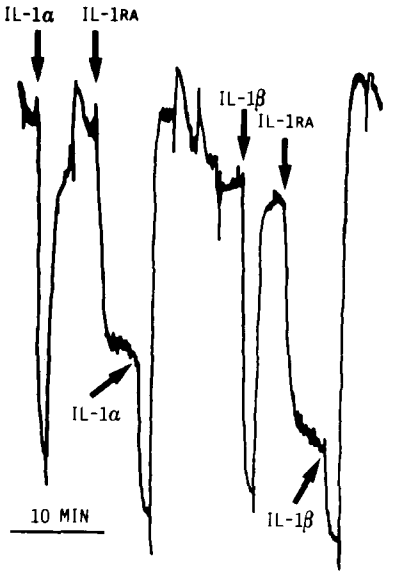

Fig. 2. Lack of antagonism by interleukin-1 receptor antagonist against interleukin-1α- or interleukin-1β-induced gastric relaxation. The figure shows relaxations induced by 60 pg/ml interleukin-1α or interleukin-1β given alone and in the presence of 100 pg/ml interleukin-1 receptor antagonist, which was added to the bath solution 10 min before. Representative tracing from 8 experiments. Washout at the plateau of interleukin-1α or interleukin-1β responses (3 min after the addition of these cytokines).

Interleukin-1 receptor antagonist was able to produce dose-dependent relaxations in strips which were not pre-contracted with 5-HT (data not shown). However, in order to facilitate our evaluation of the interleukin-1 receptor antagonist relaxing responses, all subsequent experiments were performed with 5-HT pre-contraction.

Experiments were then conducted to investigate whether the relaxant effect of both interleukin-1α and interleukin-1β could be antagonized by interleukin-1 receptor antagonist. As shown in Fig. 2, rapid strip relaxation was induced with either interleukin-1α or interleukin-1β (60 pg/ml). Strips were washed, and interleukin-1 receptor antagonist (100 pg/ml) was added to the organ baths and incubated with the tissue. The interleukin-1 receptor antagonist produced a rapid relaxation of the stomach strips, which plateaued in 10 min. Thereafter, interleukin-1α or interleukin-1β was added again, producing the same degree of relaxation previously observed in the absence of interleukin-1 receptor antagonist (Fig. 2).

Experiments were conducted with interleukin-1 receptor antagonist and BW A4c, a selective 5-lipoxygenase inhibitor which was previously shown to antagonize in a non-competitive manner interleukin-1β-induced gastric relaxation; these experiments were carried out according to a protocol described in detail elsewhere (Montuschi et al., 1994). Interleukin-1 receptor antagonist-induced gastric relaxation was dose

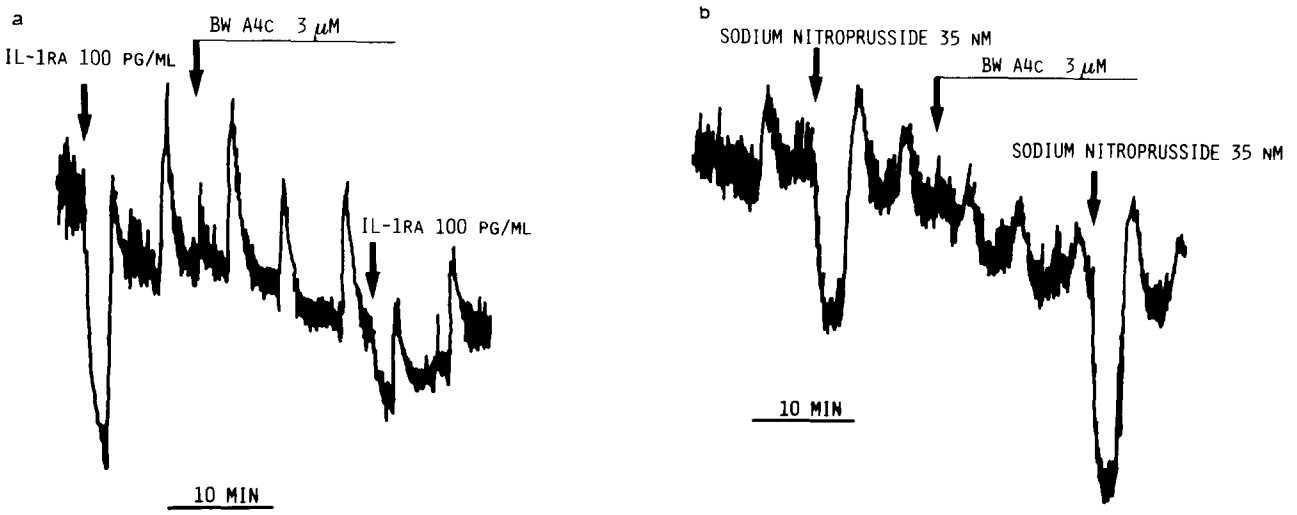

Fig. 3. The inhibitory effect of BW A4c on interleukin-1 receptor antagonist-induced gastric relaxation. (a) 3 μM BW A4c, given 15 min before the addition of interleukin-1 receptor antagonist, reduced by 70% interleukin-1 receptor antagonist-induced relaxation, compared to the relaxation in the absence of BW A4c. (b) Control experiment with 35 nM sodium nitroprusside. Representative tracings from 8 experiments.

dependently inhibited by BW A4c, with an estimated EC_{50} at 1 μM and maximal inhibition (30% of control relaxation) at 3 μM (Fig. 3a). Control relaxations induced by sodium nitroprusside were not inhibited by 3 μM BW A4c (Fig. 3b).

3.2. Release experiments

In release experiments, the two-way ANOVA analysis revealed significant differences between tissue strips, which accounts for the high variability of prostaglandin E_2 production by the different strips in basal conditions. As far as treatments were concerned, the amount of prostaglandin E_2 released by the strips in the bath solution was significantly, albeit not dose dependently, increased by interleukin-1 receptor antagonist (Table 1). Similar results were obtained with prostaglandin $F_{2\alpha}$ assays (data not shown).

Noradrenaline, sodium nitroprusside and electrical field stimulation in non-adrenergic non-cholinergic conditions, each of which induces dose-dependent (or frequency-dependent) relaxation of gastric strips, also produced an increase in prostaglandin E_2 release. However, none of these controls produced statistically significant increases (Table 1).

4. Discussion

This paper describes an unexpected effect of interleukin-1 receptor antagonist, its interleukin-1-mimicking activity on gastric fundus motility in vitro. We found that both recombinant human interleukin-1 and interleukin-1 receptor antagonist were able to produce biological responses in rat gastric tissue. In spite of the lack of species specificity, it is well accepted that interleukins of human origin display biological activities in rat tissues, as has been previously shown by our group in the present (Montuschi et al., 1993, 1994) or other (Navarra et al., 1991, 1992) experimental models.

Like interleukin-1, interleukin-1 receptor antagonist produced dose-dependent relaxation of gastric strips pre-contracted with 5-HT, though it was about 10 times less potent than interleukin-1α or interleukin-1β. A selective inhibitor of 5-lipoxygenase, BW A4c, which was previously shown to antagonize in a non-competitive manner interleukin-1-induced gastric relaxation (Montuschi et al., 1994), also inhibited relaxations induced by interleukin-1 receptor antagonist. Moreover, interleukin-1 receptor antagonist significantly increased prostaglandin E_2 production and release from gastric strips, another effect which was previously observed with interleukin-1 (Mugridge et al., 1989; Montuschi et al., 1994). The latter finding is in keeping with the observation that interleukin-1 receptor antagonist is able to increase prostaglandin E_2 production from decidual cells in vitro (Mitchell et al., 1993; Cole et al., 1993). 10 min after interleukin-1 receptor antagonist addition to the bath solution, there was still no evidence of its antagonistic action, as indicated by the lack of inhibition of relaxation induced by subsequent additions of interleukin-1α or interleukin-1β.

The agonist actions of interleukin-1 receptor antagonist in this experimental model occurred almost immediately after interleukin-1 receptor antagonist addition to the incubation medium; strip relaxation was maximal after 3 min and prostaglandin E_2 release was significantly increased within 5 min. In spite of extensive studies conducted in the last years, the very early events following receptor occupancy by interleukin-1

receptor antagonist have been poorly investigated. A paper by Dripps et al. (1991) addressed this issue, showing that interleukin-1 receptor antagonist was unable to initiate either receptor internalization or protein kinase activity (measured as phosphorylation of EGF receptors) in a murine thymoma cell line. While protein kinase was fully activated by interleukin-1α in 10–15 min, receptor internalization took place with a half-life of 1–2 h and, thus, it cannot be considered a very early signalling event. These authors showed that interleukin-1-induced protein kinase activation and receptor internalization were mediated in these cells by the activation of the 80-kDa (type-I) interleukin-1 receptor. However, the possibility cannot be ruled out that, in our model, the actions of interleukin-1 and interleukin-1 receptor antagonist could be mediated through type-II interleukin-1 receptors. In fact, the latter have been shown to mediate some effects of interleukin-1 at a gastric level, such as gastroprotection against non-steroidal anti-inflammatory drugs (Mugridge et al., manuscript in preparation).

Another line of evidence that interleukin-1 and interleukin-1 receptor antagonist share agonist activity in this experimental model comes from experiments with a specific inhibitor of 5-lipoxygenase, BW A4c (Salmon and Garland, 1991). This substance was previously

Table 1
The release of prostaglandin E_2 from rat gastric strips under basal conditions and after stimulation with graded doses of interleukin-1 receptor antagonist or other relaxing agents (noradrenaline, sodium nitroprusside or electrical field stimulation in non-adrenergic non-cholinergic conditions)

Treatments	pg PGE_2/ml	% Change vs. previous basal and statistics	
Vehicle (phosphate buffer + human serum albumin 0.2%)			
Basal	12.23 ± 1.45		
Vehicle	9.99 ± 1.22	−18.31	NS [a]
Interleukin-1 receptor antagonist			
1. Basal	12.00 ± 3.22		
Interleukin-1 receptor antagonist 10 pg/ml	33.46 ± 8.38	+179	$P < 0.02$
2. Basal	16.58 ± 4.21		
Interleukin-1 receptor antagonist 100 pg/ml	37.54 ± 8.32	+126.4	$P < 0.05$
3. Basal	15.72 ± 3.72		
Interleukin-1 receptor antagonist 1 ng/ml	40.45 ± 6.90	+157.3	$P < 0.01$
Noradrenaline			
1. Basal	24.87 ± 6.78		
Noradrenaline 10 nM	33.94 ± 8.72	+36.5	NS
2. Basal	15.15 ± 3.46		
Noradrenaline 100 nM	26.06 ± 7.36	+72	NS
3. Basal	18.96 ± 5.42		
Noradrenaline 1 μM	31.57 ± 7.40	+66.5	NS
Electrical field stimulation			
1. Basal	10.59 ± 2.75		
1 Hz	14.79 ± 5.05	+39.7	NS
2. Basal	6.34 ± 2.19		
2 Hz	11.01 ± 3.85	+73.6	NS
3. Basal	5.89 ± 1.79		
4 Hz	11.86 ± 4.26	+101.3	NS
4. Basal	5.40 ± 1.31		
8 Hz	11.68 ± 3.28	+116.3	NS
5. Basal	9.60 ± 3.34		
16 Hz	18.42 ± 5.97	+91.8	NS
Sodium nitroprusside			
1. Basal	13.81 ± 4.25		
Sodium nitroprusside 0.35 μM	22.16 ± 6.74	+60.5	NS
2. Basal	11.97 ± 3.57		
Sodium nitroprusside 3.5 μM	25.31 ± 8.31	+111.5	NS
3. Basal	11.83 ± 3.76		
Sodium nitroprusside 35 μM	27.80 ± 9.34	+135	NS

Data are expressed as pg prostaglandin E_2/ml, means ± S.E.M. of 8 observations per group. [a] NS, not significant.

shown to inhibit in a dose-dependent manner interleukin-1-induced gastric relaxation. Although this antagonism was non-competitive in nature, it turned out to be rather specific, since BW A4c also inhibited the action of tumor necrosis factor, but with a different EC_{50} (0.3 versus 1.1 μM), and it did not inhibit relaxations induced by 100 μM papaverine, 35 μM sodium nitroprusside and 0.3 μM noradrenaline (Montuschi et al., 1994). In this study we observed that BW A4c inhibits interleukin-1 receptor antagonist relaxation according to the same pattern previously observed with interleukin-1; the estimated EC_{50} was 1 μM and the maximal inhibition (30% of control relaxation) was at 3 μM.

It is well established that the actions of interleukin-1 in many tissues are accompanied by an increase in the local production of prostaglandins, which modulate or mediate some effects of the cytokine, its pro-inflammatory action in particular (Dinarello, 1991). The increase in prostaglandin E_2 synthesis induced by interleukin-1 in fibroblasts in vitro is considered by some authors to be a late event after receptor occupancy (Dripps et al., 1991). This model has been used to show that interleukin-1 receptor antagonist does not stimulate prostaglandin E_2 production in incubation experiments carried out for 16 h or more (Eisenberg et al., 1990). Furthermore, interleukin-1 receptor antagonist was able to inhibit interleukin-1-induced prostaglandin E_2 production in many cell lines (Arend et al., 1990). In contrast, Mitchell et al. (1993) have recently shown that interleukin-1 receptor antagonist is able to produce a dose-dependent increase in prostaglandin E_2 production by decidual cells in vitro. Further evidence that interleukin-1 receptor antagonist may act as an agonist of prostaglandin E_2 production from decidual cells has been recently provided by Cole et al. (1993). In keeping with the latter observations, the present findings show that interleukin-1 receptor antagonist possesses intrinsic agonist activity on prostaglandin E_2 release from rat gastric strips in vitro. Moreover, these data, together with previous evidence (Montuschi et al., 1994), show that an increase in prostaglandin synthesis can also occur very rapidly after interleukin-1 receptor occupancy. The early increase in prostaglandin release is not specific for gastric tissue, since it has also been observed in the rat hypothalamus in vitro, as soon as 20 min after the addition of interleukin-1β to the incubation medium (Navarra et al., 1992).

How can the agonist action of interleukin-1 receptor antagonist be explained? We could exclude the possibility that these effects of interleukin-1 receptor antagonist were due to a contaminant in our preparation, since the same batch of interleukin-1 receptor antagonist used on gastric strips did not increase prostaglandin E_2 production from rat hypothalamic explants (manuscript submitted). Mugridge et al. (1989, 1991) have found that interleukin-1 caused no changes in strip motility in a Ca^{2+}-free Krebs solution, and that pre-treatment of the strips with the cytokine resulted in a potentiation, rather than an inhibition, of the contractile response induced by subsequent addition of Ca^{2+} to the medium. These authors did not investigate whether interleukin-1 receptor antagonist had any relaxing effect in this experimental model. The latter model, albeit similar, is not comparable to that used in the present study, because of the lack of Ca^{2+} in the incubation medium when interleukin-1 was given. In fact, we have demonstrated that the gastric relaxation induced by interleukin-1β (and presumably interleukin-1 receptor antagonist) is caused by the Ca^{2+}-dependent activation of a phospholipase C specific for phosphatidylcholine (Montuschi et al., 1994). Therefore, in the absence of Ca^{2+}, this mechanism was not active in the experiments by Mugridge et al. It should also be pointed out that, in a Ca^{2+}-free medium, gastric strips are completely relaxed and no further relaxation can be elicited by any of the known relaxing agents.

A hypothesis to explain the agonist activity of interleukin-1 receptor antagonist is not substained at present by experimental evidence. One can speculate that the very early event after receptor occupancy by interleukin-1 receptor antagonist might be a rapid and massive over-stimulation, which causes desensitization of signalling mechanisms and unresponsiveness to full agonists. As stated above, the early events occurring after receptor occupancy by interleukin-1 receptor antagonist have not been investigated in detail, especially as far as the type II of interleukin-1 receptors are concerned. Alternatively, interleukin-1 receptors in the gastric fundus might be activated by regions of the interleukin-1 receptor antagonist molecule with high homology with interleukin-1. In this regard, several studies have shown a complex structure-activity relationship for interleukin-1. In fact, different biological activities (adrenocorticotropin release, prostaglandin E_2 induction, fibroblast growth, T-cell stimulation) are related to different parts of the primary structure of the molecule (Dinarello, 1991). The ability of interleukin-1 to inhibit gastric motility might be associated with a part of the primary structure of the protein in the region with high homology with the interleukin-1 receptor antagonist, which might therefore share with the former agonist activity in this model.

In conclusion, in this paper we have shown that interleukin-1 receptor antagonist shares with interleukin-1 the ability to induce dose-dependent relaxation of rat gastric fundus strips. This effect is antagonized by a specific inhibitor of 5-lipoxygenase, BW A4c, and is accompanied by an increased release of prostaglandin E_2 into the bath solution. These findings are difficult to explain on the basis of the present

knowledge of the biology of interleukin-1 receptor antagonist.

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