Identification and functional importance of IL-1 receptors on rat parietal cells

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Scheppe, Wolfgang, Kerstin Dehne, Hedda Hermuth, Klaus Pfeffer, and Christian Prinz. Identification and functional importance of IL-1 receptors on rat parietal cells. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1094–G1105, 1998.—We studied the expression of interleukin-1 (IL-1) receptors and the effect of IL-1β on the function of highly enriched (≥97%) rat parietal cells. RT-PCR of parietal cell poly(A)+ RNA with primers specific for the rat IL-1 receptor revealed a single 547-kb PCR product highly homologous to the published sequence of the IL-1 receptor. Northern blot analysis of poly(A)+ RNA of rat parietal cells and brain revealed a single RNA species of 5.7 kb. Cytochemistry of parietal cell IL-1 receptor was performed with biotinylated recombinant human IL-1β, visualized by avidin-coupled fluorescein. Corresponding to the high degree of parietal cell enrichment, 95% of the cells stained positive. Basal H+ production ([14C]aminoxyacetic accumulation) was not changed by IL-1β (0.25–100 pg/ml) nor was the response to histamine or carbachol when added simultaneously with the cytokine. However, when parietal cells were preincubated with IL-1β (0.5–5 pg/ml) for 10 min before the addition of histamine or carbachol, the response to these secretagogues was reduced by 35 and 67%, respectively. Inhibition by IL-1β was fully reversed by the human recombinant IL-1 receptor antagonist. Preincubation of parietal cells with IL-1β failed to alter histamine-stimulated cAMP production but markedly inhibited carbachol-induced formation of d-myo-inositol 1,4,5-trisphosphate. In fura 2-loaded, purified parietal cells, 10 min preincubation with IL-1β dramatically reduced the initial transient peak elevation of intracellular Ca2+ concentration in response to carbachol. We conclude that rat parietal cells express IL-1 receptors mediating inhibition of H+ production. The antisecretory effect of IL-1β may contribute to hypoaclidity secondary to acute Helicobacter pylori infection or during chronic colonization by H. pylori: preferring the fundic mucosa.

interleukin-1; interleukin-1 receptor antagonist; hydrogen production; adenosine 3′,5′-cyclic monophosphate; d-myo-inositol 1,4,5-trisphosphate; cytosolic calcium; fluorescence microscopy; fura 2-acetoxymethyl ester

THE PROINFLAMMATORY cytokine interleukin-1β (IL-1β) is a polypeptide product of monocytes and activated macrophages released at numerous sites of inflammation. In the gastric mucosa, infection with Helicobacter pylori is followed by an intensive infiltration of inflammatory cells releasing substantial amounts of cytokines, among them IL-1β. Intact H. pylori as well as soluble H. pylori surface proteins induce the production of IL-1 from human monocytes (22). This multifunctional cytokine not only serves as an important mediator of the inflammatory response of the host by inducing IL-2 secretion and IL-2 receptor expression of helper T cells but also modulates the biological function of different gastric epithelial cell types. In cultured G cells from the rat antrum the cytokine stimulated gastrin release (55). In resting rat fundic enterochromafﬁn-like (ECL) cell cultures, IL-1β moderately stimulated the release of histamine, whereas 20 min preincubation of these cells with the cytokine markedly inhibited gastrin-stimulated histamine secretion (31). Moreover, after 24 h of incubation of rat ECL cell cultures with IL-1β, the rate of apoptotic cells was more than doubled, an effect partially mediated by the inducible form of nitric oxide (NO) synthase and by NO formation (30). Thus, by modulating the physiological function of gastric epithelial cells, IL-1β potentially contributes to hypergastrinemia and decreased gastric mucosal content of histamine, conditions frequently observed in H. pylori gastritis.

Effects of IL-1β on parietal cells have been investigated in less detail, although such experiments have been proposed earlier based on animal studies in vivo (32). IL-1β inhibited gastric acid secretion not only via central nervous mechanisms, interacting at hypothalamic and medullar structures, but also, after intravenous administration, via peripheral mechanisms (19, 35, 36, 37, 52). On the basis of these data, it has been speculated that IL-1β might interact with parietal cell receptors to inhibit gastric acid secretion (32).

In fact, IL-1α, a 27% sequence-homologous peptide almost functionally equivalent to IL-1β, inhibited histamine- and carbachol-stimulated H+ production of isolated, highly enriched canine parietal cells, an effect not mediated by endogenous PG formation (28). These data yielded functional evidence that parietal cells express the IL-1 receptor (IL-1R). In general, binding to this receptor is determined by the carboxy terminus of IL-1, which is highly homologous between IL-1α and IL-1β. This homology is probably the reason for the nearly identical biological activities of both cytokines (7, 23) and provided a rationale for a recent study (1) in rabbit parietal cells. In these cells (1), IL-1β was more effective in inhibiting H+ production stimulated by carbachol and gastrin than by histamine. This inhibitory effect of IL-1β was not mediated by changes in radioligand binding to histamine H2 or gastrin/CCKB receptors. Rather, IL-1β appeared to interact with postreceptor mechanisms. These, however, were not

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studied directly but rather by measuring the effects of IL-1β on H⁺ production in response to specific postreceptor agonists or inhibitors (1).

Therefore, it was the aim of the present study to directly investigate mechanisms mediating the inhibitory effect of IL-1β on parietal cell function. Using highly enriched rat parietal cells, we identified IL-1R by molecular biological and immunocytochemical techniques. Moreover, we confirmed in this cell type the inhibitory effect that IL-1β, by interacting with these receptors, exerts on H⁺ production. Finally, we found direct evidence that IL-1β modulates parietal cell function by interfering with Ca²⁺/phospholipid- rather than cAMP-dependent signaling pathways that mediate the key secretagogue effects of carbachol and histamine, respectively (50).

MATERIALS AND METHODS

Materials. All reagents were of analytic grade and were purchased from the indicated suppliers. Carbachol, nitro blue tetrazolium, and ionomycin were obtained from Sigma (Munich, Germany). Quicksint 2000 scintillation cocktail and Biolutes S tissue stabilizer were from Zinsser Analytic (Frankfurt, Germany), and guanidium isothiocyanate was from GIBCO (Berlin, Germany). BSA, DMEM containing HEPES and glutamine, IBMX, HEPES, and histamine were obtained from Serva (Heidelberg, Germany). 1,4-Dithiothreitol, Pronase E, trypan blue, Na₂EDTA, and all buffer constituents were from Merck (Darmstadt, Germany). Fura 2-AM was obtained from Molecular Probes (Eugene, OR), and recombinant human IL-1β [10 pg = 1.87 World Health Organization (WHO) units] and recombinant human IL-1R (rhIL-1R) antagonist were from R & D Systems (Abingdon, United Kingdom). Cell-Tak was from Becton Dickinson Labware (Bedford, MA).

Media. Medium A contained 0.5 mM Na₂HPO₄, 1.0 mM Na₂HPO₄, 20 mM NaHCO₃, 70 mM NaCl, 5 mM KCl, 11 mM glucose, 50 mM HEPES buffer, 1 mM Na₂EDTA (pH 7.8), and BSA (10 mg/ml). Medium B had a similar composition; EDTA was substituted with 1.0 mM CaCl₂ and 1.5 mM MgCl₂. Medium C consisted of 340 mM NaCl, 1.2 mM MgSO₄, 10 mM CaCl₂, 15 mM HEPES (pH 7.4), 11 mM t-glucose, 0.1% BSA, and 0.5 mM dithiothreitol.

Gastric mucosal cell isolation. Gastric epithelial cells were isolated as described previously (29) with slight modifications. Five female nonfasted Sprague-Dawley rats (200 g, Charles River, Sulzfeld, Germany) per experiment were killed by cervical dislocation. After excision, the stomachs were rinsed with 0.9% saline and everted to form sacs with the mucosa facing outward and the serosa facing inward. Three milliliters of Pronase E solution (1.3 mg/ml) were injected into the sac lumina. The stomachs were incubated at 37°C for 35 min in oxygenated medium A. Superficial mucosal cells were discarded after incubating the stomachs for 10 min in oxygenated medium B. After another 30 min in oxygenated medium A, cells for elutriation were harvested by 8 min of stirring in medium B and collected by centrifugation at 800 rpm for 5 min (IEC 6000 B centrifuge, Nunc, Bedfordshire, United Kingdom) (fraction 0). Subsequently, the stomachs were placed again in medium A for another 20 min, followed by two more cell collection steps in medium B (for 8 min). Collected cells (4.5 ± 0.7 × 10⁶ cells/preparation) were resuspended in medium C.

Parietal cell enrichment. The crude cell suspension was separated according to increasing cell diameters by counter-flow elutriation (rotor E-68, equipped with standard chamber; centrifuge J-2–21M/E; Beckman Instruments, Glenrothes, United Kingdom) as previously described (44). Briefly, the crude suspension of freshly isolated cells (fraction 0) was loaded into the rotor at a flow rate of 16 ml/min and a rotor velocity of 2,300 rpm. Erythrocytes, cell fragments, and cells with diameters <14 µm were removed by washing at 40 ml/min and 2,000 rpm. Thereafter, a cell fraction was collected at 62 ml and 2,000 rpm. This fraction consisted of 82% parietal cells (nitro blue tetrazolium staining).

For further enrichment of parietal cells, the elutriated fraction was loaded on a density gradient (60% Percoll, 40% buffer) performed by ultracentrifugation (20,000 g, 20°C, 45 min). After 20 min of centrifugation (2,000 g, 20°C), almost pure parietal cells (97–100%) were collected at the interface between Percoll and buffer. The percentage of parietal cells was routinely determined by nitro blue tetrazolium staining of each cell preparation studied. Beyond that, we performed additional staining with the mouse monoclonal antibody 4A6, directed against the α-subunit of H⁺,K⁺-ATPase (kindly donated by J. L. Cabero, Gothenburg, Sweden) in seven cell preparations. Viability of enriched parietal cells was determined by trypan blue exclusion and exceeded 95%.

Parietal cell culture. Parietal cell culture was performed as described previously (4). Enriched parietal cells were washed three times by low-speed centrifugation in serum-free culture medium (1:1 Ham's F-12 medium/DMEM with HEPES and L-glutamine, without bicarbonate) supplemented with insulin (5 µg/ml), transferrin (5 µg/ml), sodium selenite (5 ng/ml), hydrocortisone (4 ng/ml), epidermal growth factor (EGF) (25 ng/ml), gentamicin (10 mg/100 ml), and cell culture grade BSA (2 mg/ml). For removal of contaminating fibroblasts, cells were preattached toflasks coated with 10% calf serum in PBS (30 min at 37°C). After preattachment, 5 × 10⁶ parietal cells were cultured on coverslips precoated with Cell-Tak (3.5 µg/cm²). After 48 h in primary culture, the purity of parietal cells was 98–100% as determined by staining with nitro blue tetrazolium and the mouse monoclonal antibody 4A6. Cell viability (trypan blue exclusion) exceeded 95%.

Poly(A)⁺ RNA preparation. Total cellular RNA was prepared by disrupting acutely isolated, highly enriched rat parietal cells with guanidium isothiocyanate and cesium chloride. Poly(A)⁺ RNA was purified by oligo(dT) cellulose, according to standard procedures (33). The RNA yield was quantified by determining the optical density at 260 nm with a Beckmann DU 640 spectrophotometer.

RT-PCR analysis of rat IL-1R. After a DNase I (GIBCO BRL Life Technologies, Gaithersburg, MD) digestion, parietal cell poly(A)⁺ RNA (100 ng) was reverse transcribed into the corresponding single-strand cDNA with oligo(dT)₁₅ primers, using a first-strand cDNA synthesis kit (AMV, Boehringer Mannheim). PCR was performed in Taq DNA polymerase buffer (final concn, 1.5 mM MgCl₂) and 1 unit Taq DNA polymerase (GIBCO BRL), using the Primerset PCR kit (Biometra, Göttingen, Germany) and oligonucleotide primers under the following conditions: hot start, 94°C for 5 min and 85°C for 5 min; initial step (1 cycle), 62°C for 1 min and 72°C for 3 min; repeating steps (30 cycles), 94°C for 1 min, 62°C for 1 min, and 72°C for 3 min; and extension step (1 cycle), 94°C for 1 min, 62°C for 1 min, and 72°C for 7 min. The sense primer for subunit I of the rat IL-1R was 5'-CC TTT GCC CCA CGT CCT ACA CAT ACC-3', and antisense primer sequence was 5'-CGG GGA AGA AAA TCA GAG CAG GAG-3'. The calculated size of the PCR product was 547 bp. The sequence of the PCR product was verified by a commercial institute (MediGene, Martinsried, Germany).
Northern blot. To produce a cDNA probe specific for type I of the rat IL-1R, a 546-bp fragment of the receptor was amplified by PCR, directly cloned into pPCR 2.1 (Invitrogen, Leek, The Netherlands), and transformed into competent bacteria (DH5α). Plasmid DNA was extracted from bacteria (using Qiagen columns, according to manufacturer's suggested protocol). The IL-1 insert was removed from the plasmid by EcoRI digestion, separated by agarose gel electrophoresis (0.8% agarose), and purified with glass beads (GeneClean II, Dianova, Hamburg, Germany). 32P labeling of the cDNA was performed by random priming (12). The cDNA probe corresponded to bp 956–1502 of the mRNA of rat type I IL-1R [GenBank accession no. M95578; base count according to Hart et al. (17)].

Poly(A)+ RNA from acutely isolated rat parietal cells and from rat brain (Clonetech, Heidelberg, Germany) was separated in parallel on a 1.0% formaldehyde-agarose gel and blotted onto a nylon membrane (ICN, Irvine, CA). Hybridization of the blot was carried out at 65°C in hybridization buffer containing 1 × 106 cpm/ml of the specific probe. After washing at high stringency [2.0% SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 1% SDS at 58°C], filters were exposed to Kodak X-AR films (Eastman Kodak, Rochester, NY) at −70°C for 2 weeks. Immunolabeling. Highly enriched parietal cells were grown in primary culture (48 h) on Cell-Tak-coated glass slides. Biotinylated rhIL-1β (2.5 µg/ml, 30 µl/slide; R & D Systems) was applied to the cells (1 h at 4°C). This specific ligand is designed to quantitatively determine the percentage of cells bearing the cytokine receptor within a given population. For visualization, avidin-coupled fluorescein (10 µl/slide; R & D Systems) was applied in the dark (30 min at 4°C). After washing with buffer, cells were immediately visualized under a fluorescence microscope (Axiovert 100 TV, Zeiss, Jena, Germany; magnification, ×40; excitation wavelength, 480 nm). For negative control, biotinylated rhIL-1β was incubated with an 800-fold excess of polyclonal goat IgG anti-human IL-1β antibody (R & D Systems) (15 min at 20°C) before being applied to the cultured cells.

[3H]Aminopyrine accumulation. Accumulation of the weak base [3H]aminopyrine served as a quantitative index of parietal cell H+ production (49). Our modifications of the original procedure have been described previously (41, 42). Acutely isolated highly enriched parietal cells (5 × 106 cells/ml) were preincubated for 2 h at 37°C in DMEM in the absence of test agents. Next, 400 µl of the cell suspension (3 × 106 cells/ml) were incubated together with [3H]aminopyrine (0.04 µCi/tube) in flat-bottomed plastic vials in a shaking bath (37°C) in the absence or presence of IL-1β (15 min at 20°C) before being applied to the cultured cells.

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IL-1β AND PARIETAL CELL FUNCTION

RESULTS

Parietal cell IL-1R: PCR, Northern blot analysis, and immunolabeling. RT-PCR was performed with single-strand cDNA derived from reverse transcription of rat parietal cell poly(A)+ RNA using primers specific for the known sequence of type I of the rat IL-1R. The reaction yielded a single primer product at the expected size of 547 bp (n = 5 independent experiments; Fig. 1). The product was cut out, separated, and sequenced and revealed complete homology with the known receptor sequence of type I of the rat IL-1R. The reaction revealed complete homology with the known receptor product was cut out, separated, and sequenced and yielded a single primer product at the expected size of 5.7 kb (Fig. 2).

Free cytosolic Ca2+ concentration ([Ca2+]i) was determined according to the equation [Ca2+]i = Kd(R - Rmin/Rmax - R)[Dlow/Dhigh], where Kd is the dissociation constant for fura 2-AM (224 nM) and R the measured ratio. Rmin is the limiting value of R when all the indicator is in the Ca2+-free form (low standard, 2 mM EGTA (pH 7.5)), Rmax is the limiting value of R when all the indicator is saturated with Ca2+ (high standard, 10 μM ionomycin). Dlow/Dhigh is the ratio of measured fluorescence intensity when all the indicator is Ca2+-free, to the intensity when all the indicator is Ca2+ bound (both measurements performed at 380 nm). This equation was used as described previously (16, 57) to relate the ratio of chosen 334/380 pixel pairs to [Ca2+]i.

Statistics. Data were expressed as means ± SE from four to seven independent experiments, each performed in quadruplicate (14C]aminopyrine accumulation) or duplicate (cAMP and IP3 formation). On the basis of the absolute values, ANOVA for multiple determinations was performed. Student's t-test for unpaired data was performed as a post hoc test for calculation of statistically significant differences between individual treatments. P ≤ 0.05 was considered significant.

The chamber was continuously perfused (2 ml/min medium C) without or with the indicated test agents (carbachol or without IL-1β). After each exposure to test agents, cells were washed with Ca2+-free medium C for 5 min, followed by 5 min with Ca2+-containing medium C to allow for restoration of cellular Ca2+ stores. During exposure to test agents, cells were observed under an Axiovert 100 TV microscope (Zeiss) in the epifluorescence mode using a dichroic filter (395 nm). Excitation by alternating wavelengths (334 nm, 380 nm) was performed by a motorized filter wheel. The image field was monitored by an Attofluor charge-coupled device camera (Zeiss). Image pairs were captured (~1 s apart) under the control of Attofluor/Ratio Vision software (Atto Instruments, Rockville, MD) and stored as digitized 256 gray level images.

Effect of IL-1β on parietal cell [14C]aminopyrine accumulation. In the presence of the phosphodiesterase inhibitor IBMX (10−4 M), basal [14C]aminopyrine accumulation in acutely isolated, highly enriched rat parietal cells was stimulated 11.7-fold by 3 × 10−6 M histamine. This submaximal concentration elicited 57 ± 7% of the maximal response to 10−4 M histamine, an effect quite similar to the stimulation by 10−5 M carbachol (see Fig. 5A). IL-1β (1.0–1,000.0 pg/ml) had no effect on basal H+ production (Table 1). When IL-1β was added to the cells simultaneously with 3 × 10−6 M histamine, the cytokine failed to affect the response (Table 2). However, when the cells were preincubated for 10 min in the presence of IL-1β, the cytokine moderately inhibited the stimulation by 3 × 10−6 M histamine.
histamine; maximal inhibition by 35% was observed with 2.5 pg/ml IL-1β (P < 0.05) (Fig. 4A). Preincubation with 2.5 pg/ml IL-1β for 20 and 30 min did not result in more effective inhibition (Table 2). When IL-1β (0.5–5.0 pg/ml) was added 10 min before the maximally effective concentration of histamine (10⁻⁴ M), the cytokine failed to induce significant inhibition (Table 3).

Parietal cells were treated for 10 min with the rhIL-1R antagonist at 100, 250, and 500 pg/ml. They were then incubated for another 10 min with the
Table 1. Effect of IL-1β on basal [14C]aminopyrine accumulation in purified rat parietal cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Medium</th>
<th>Basal</th>
<th>1.0</th>
<th>10.0</th>
<th>100.0</th>
<th>1,000.0</th>
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<tr>
<td></td>
<td>IBMX + 1 x 10^{-3} M Ca^{2+}</td>
<td>2.9 ± 0.6</td>
<td>2.6 ± 0.9</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.8</td>
<td>2.7 ± 0.7</td>
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<tr>
<td></td>
<td>3 x 10^{-3} M Ca^{2+}</td>
<td>3.1 ± 0.5</td>
<td>2.9 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.7</td>
<td>3.2 ± 0.5</td>
</tr>
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Values are means ± SE from n = 5 independent cell preparations and are expressed as [14C]aminopyrine accumulation ratios. Effect of interleukin-1β (IL-1β) on basal [14C]aminopyrine accumulation in acutely isolated highly purified rat parietal cells is shown. Cells were incubated for 10 min with vehicle (basal) or IL-1β at increasing concentrations. IBMX was given at a concentration of 10^{-4} M.

The effect of IL-1β on histamine-stimulated H^+ production was studied in the absence of IBMX in Ca^{2+}-supplemented medium. Also under these conditions, IL-1β had no effect on basal H^+ production (Table 1). At the maximally effective concentration, 10^{-5} M carbachol stimulated [14C]aminopyrine accumulation 11.6-fold above basal, a response quantitatively quite similar to that to 3 x 10^{-6} M histamine (see Fig. 4A).

Similar to the experiments with histamine, addition of IL-1β (2.5 pg/ml) simultaneously with carbachol did not affect the cholinergic stimulation (Table 2). However, when the cells were preincubated with IL-1β 10 min before the addition of carbachol, the cytokine inhibited the response in a concentration-dependent manner (Fig. 5A). Maximal inhibition by 67 ± 6% was obtained with 2.5 pg/ml IL-1β (P < 0.01). Preincubation with 2.5 pg/ml IL-1β for 20 and 30 min resulted in a slightly less effective inhibition compared with the response after 10 min of preincubation with the cytokine (Table 2). After 10 min of preincubation with rhIL-1R antagonist (100, 250, or 500 pg/ml), parietal cells were preincubated for another 10 min with the maximally effective concentration of IL-1β (2.5 pg/ml), before being stimulated with 10^{-5} M carbachol. IL-1β inhibited carbachol-
stimulated [14C]aminopyrine accumulation by 77 ± 6%. This inhibitory effect was gradually overcome by preincubation with increasing concentrations of the rhIL-1R antagonist, which at 250 and 500 pg/ml completely restored the response to carbachol (Fig. 5B).

Effect of IL-1β on parietal cell cAMP and IP3 production. cAMP production of acutely isolated highly enriched rat parietal cells was studied in the presence of the phosphodiesterase inhibitor IBMX (10−4 M). The submaximally effective concentration of 10−5 M histamine was used for stimulation. In control cells preincubated in the absence of IL-1β, histamine (10−5 M) stimulated cAMP production 5.4-fold above the basal rate already after 3 min. This response increased to a sevenfold stimulation after 6 and 10 min of exposure to histamine. Cells of the identical preparations were preincubated uniformly in the presence of 2.5 pg/ml IL-1β for 10 min. Thereafter, cells were stimulated by 10−5 M histamine for 3, 6, and 10 min, respectively. Compared with control cells, there was only a trend toward a small inhibitory effect of IL-1β at 3 min of exposure to histamine (P > 0.05). At 6 and 10 min of exposure to histamine, preincubation with the cytokine did not affect the response to the agonist (Fig. 6). Thus preincubation of the cells with IL-1β at the identical concentration and for the identical time interval yielded inhibition of histamine-stimulated [14C]aminopyrine accumulation failed to significantly reduce cAMP production in response to histamine.

At 10−4 M, carbachol stimulated IP3 formation of acutely isolated highly enriched rat parietal cells 2.9-fold above basal under control conditions, i.e., after preincubation in the absence of IL-1β. However, when cells were preincubated with 2.5 pg/ml IL-1β 10 min before the addition of carbachol, the cytokine markedly inhibited the response to carbachol by 75 ± 8% (P < 0.01 vs. control cells' response to carbachol; Fig. 7). Thus, under identical conditions, IL-1β inhibited [14C]aminopyrine accumulation and IP3 formation in response to a maximally effective concentration of carbachol.
carbachol elicited a fluorescence signal in 93 ± 9% of the cells. Carbachol elicited a biphasic response, an initial transient followed by a lasting plateau phase. The initial peak response was observed within 40 s on stimulation with carbachol, and the plateau phase decayed to the baseline after carbachol and Ca²⁺ were removed from the perfusion medium (Fig. 8A). After recovery of the cells in Ca²⁺-containing medium, 10⁻⁴ M carbachol was added to the perfusion medium for a second period of stimulation yielding the identical biphasic response of [Ca²⁺]ᵢ, i.e., initial peak transient and sustained phase as observed after the initial stimulation of the identical cells (Fig. 8A). Thus, under the conditions used, parietal cells responded reproducibly and identically to two consecutive challenges with carbachol.

In the cells chosen to study the effects of IL-1β, initial stimulation with carbachol elicited the identical response as in control cells (Fig. 8B). After the recovery phase, IL-1β (2.5 pg/ml) was added to the perfusion medium for 10 min in the absence of carbachol. The cytokine exerted no detectable effect on the basal level of [Ca²⁺]ᵢ. In contrast, on subsequent stimulation with carbachol in the presence of IL-1β, the peak response to the cholinergic agonist was dramatically reduced compared with the initial response of this cell and to the initial and second stimulation in control cells.
last duration of plateau phase after the reduced initial peak, however, was not affected by IL-1β (Fig. 8B).

**DISCUSSION**

The present study demonstrates that rat parietal cells express functionally relevant IL-1R mediating impairment of H+ production. The cytokine inhibited carbachol-stimulated parietal cell function more effectively than the response to histamine. The cellular mechanisms mediating the inhibitory effect of IL-1β involved impairment of the IP3-dependent increase in cellular [Ca2+]i, rather than a decrease of the production of cAMP.

Characterization of the parietal cell IL-1R revealed similarities with IL-1R expressed by rat fundic ECL cells (31). The parietal cell IL-1R is type I (11) as evidenced by the RT-PCR and Northern blot analysis in this study. Type I IL-1R is an 80-kDa transmembrane protein mediating the biological effects of IL-1β. On the other hand, the type II receptor is a 68-kDa membrane protein without an apparent signaling function. Rather, the type II receptor appears to act as a decoy target for IL-1, inhibiting IL-1 activities by preventing the binding of this cytokine to type I receptors. We performed a database search using the TBLASTN algorithm. This search revealed no sequence homology allowing for cross hybridization of our IL-1R type I-specific probe with type II mRNA. However, the potential expression of type II IL-1R by rat parietal cells was not examined in the present study. Our Northern blot analysis of parietal cell RNA with a cDNA probe specific for the rat IL-1R yielded a signal of a size quite similar to that initially determined in mouse T cells (~5.0 kb; Ref. 46).

The parietal cell IL-1R is of functional relevance and mediates inhibition of H+ production. Reversal of the inhibitory response by the IL-1R antagonist supported the view that inhibition by IL-1β is a specific, receptor-mediated effect. The IL-1R antagonist is a naturally occurring protein inducible in many cell types and constitutively expressed in intestinal epithelial cells. The IL-1R antagonist lacks a site binding a receptor accessory protein necessary for inducing biological effects mediated via type I IL-1R. Thus, on binding to these receptors, the IL-1R antagonist exerts no agonist activities but rather inhibits the binding of IL-1 and the subsequent biological effects. In our rat parietal cell system a 100-fold excess of rhlIL-1R antagonist over the maximally effective concentration of IL-1β was sufficient to completely prevent the inhibitory response to the cytokine. This ratio is compatible with the view that the parietal cell IL-1R mediating inhibition of H+ production is type I, since the affinity of the antagonist to type II receptors is 1,000-fold lower than that of IL-1 (9, 14).

The potency at which IL-1β acted on our rat parietal cell system was similar to that by which the cytokine modulated the growth of the murine helper T cell line D10.G4.1 (51). Moreover, the IL-1β concentrations effective at rat parietal cell IL-1R closely resembled those at which the cytokine modulated hormone secretion from cultured rat pituitary cells (2) and impaired the functional characteristics of cultured rat pancreatic islets (10, 34, 47), insulinoma INS-1 cells (20), and fundic ECL cells (30, 31). On the other hand, 1,000-fold higher concentrations of IL-1β were required to stimulate gastrin release from rabbit antral G cells (55) or to inhibit rabbit parietal cell H+ production (1). Finally, IL-1β acted 200,000-fold more potently on our rat parietal cells than IL-1α did on canine parietal cells (28). This suggests that, at parietal cell IL-1R, IL-1β may be the functionally more important agonist compared with IL-1α.

The efficacy with which in the present study IL-1β inhibited rat parietal cell H+ production was quite similar to that with which the cytokine reduced histamine release from rat fundic ECL cells (31) and H+ production by canine (IL-1α; Ref. 28) and rabbit parietal cells (IL-1β; Ref. 1). Moreover, the present study demonstrates that, in inhibiting submaximally stimulated rat parietal cell function, IL-1β is as effective as PGE2 and somatostatin, which are established endogenous inhibitors of H+ production (40, 43, 45). However, in inhibiting fully stimulated H+ production in response to 10−8 M histamine plus IBMX, we found IL-1β to be less effective than PGE2 and somatostatin. In accordance with recent experiments in rabbit parietal cells (1), we observed that IL-1β inhibits rat parietal cell H+ production in response to carbachol more effectively than in response to histamine. In canine parietal cells IL-1α was more effective in inhibiting the response to histamine alone than the potentiating interaction between histamine and IBMX (28). It has to remain open whether this holds also for isolated rat parietal cells, which, in the absence of IBMX, fail to respond to histamine (42).

Inhibition of parietal cells by IL-1β depended on preincubation of the cells with the cytokine 10 min before addition of the stimulus. Apparently, IL-1β alters the signaling machinery of rat parietal cells in a manner that reduces the efficacy of subsequent, not of simultaneous, stimulation. In our parietal cell system, induction of this process required 10 min. Preincubation with IL-1β for longer time intervals resulted in less effective inhibition. The complexity of signaling mechanisms at which IL-1β may interfere to inhibit H+ production is still unresolved. However, our data shed some light on the effects of IL-1β on essential signaling pathways of parietal cells.

Key stimuli of the parietal cell function are histamine and ACh. They interact with H2 and M3 receptors governing cAMP/protein kinase A- and phosphatidyl inositol/Ca2+-dependent signaling pathways, respectively (50). In this study, our finding that IL-1β inhibited rat parietal cell H+ production in response to carbachol more effectively than that stimulated by histamine suggested that the cytokine interferes with Ca2+/phospholipid- rather than with cAMP-dependent signaling pathways. In fact, we observed that in rat parietal cells IL-1β had no significant effect on histamine-stimulated cAMP production. In contrast, we found that the cytokine substantially inhibited carbachol-induced formation of IP3. In parietal cells, this
second messenger mediates the M₃ receptor-dependent peak increase in [Ca²⁺]. Accordingly, our analysis of single parietal cells by fluorescence microscopy revealed a substantial reduction of this peak. Thus we conclude that interfering with the IP₃-dependent peak increase in [Ca²⁺] is an essential mechanism by which IL-1β inhibits rat parietal cell function.

In contrast to the present study, a recent study (1) in rabbit parietal cells did not directly investigate IL-1β effects on intracellular signaling mechanisms but rather drew indirect conclusions from [¹⁴C]aminopyrine accumulation in response to postreceptor agonists and inhibitors. In that study (1), inhibition by IL-1β of forskolin- but not of dibutylcAMP-stimulated H⁺ production suggested that the cytokine interfered at the level of cAMP production rather than at the protein kinase A or subsequent signaling steps. The inhibitory IL-1β effect on histamine- or forskolin-induced [¹⁴C]aminopyrine accumulation was reversed by pertussis toxin and herbimycin, suggesting that the cytokine interfered at G proteins and tyrosine kinases governing H₂ receptor-dependent cAMP production (1). This interpretation is at variance with our present finding that IL-1β had no effect on histamine-stimulated cAMP production. However, inhibition by IL-1β of rabbit parietal cell H⁺ production in response to the Ca²⁺ ionophore A-23187 (1) fits our conclusion that the cytokine interferes with Ca²⁺/phospholipid-dependent signaling. We propose that in parietal cells, interference with this pathway is the key effect by which IL-1β inhibits primarily H⁺ production in response to carbachol. By interfering with the IP₃-dependent peak increase in [Ca²⁺], IL-1β might impair the potentiating cross talk between Ca²⁺/phospholipid- and cAMP-dependent transduction. Therefore, IL-1β may also inhibit, to a minor degree, cAMP-mediated H⁺ production in response to histamine.

There appears to exist no uniform signal-response transduction secondary to IL-1R activation. Although IL-1 reduced cAMP production in a pertussis toxin-sensitive manner in rat pancreatic islet cell cultures (47), the cytokine had no effect on cAMP formation in J urkat (6) and U-373 MG human astrocytoma cells (4) and increased cAMP production and protein kinase A activity in human FS-4 fibroblasts (58). Breakdown of membrane inositol phosphates and IP₃ formation were not affected by IL-1 in the human leukemia-derived HSB-2 subclone (26), J urkat (6), and U-373 MG human astrocytoma cells (4), while the cytokine induced IP₃ formation in mouse peritoneal macrophages (56) and renal mesangial cells (8). IL-1β did not induce Ca²⁺ influx in the human leukemia-derived HSB-2 subclone (26) and J urkat cells (6). In this cell type IL-1 increased the synthesis of phosphatidylinerine, a necessary cofactor for the activation of protein kinase C (6). However, the cytokine lacked an effect on protein kinase C in human FS-4 fibroblasts (58) and the human leukemia-derived HSB-2 subclone (26). PGE₂ formation was induced by IL-1β in renal mesangial cells (8). On the other hand, indomethacin failed to prevent inhibition by IL-1α of H⁺ production in canine parietal cells (28), arguing against endogenous PGs as mediators of IL-1 effects on this cell type. IL-1β induced NO formation in renal mesangial cells (8) and rat fundic ECL cell cultures (31). Moreover, in the latter cell type (31), in J urkat cells (6), and cultured rat pancreatic islets (15), IL-1β induced cGMP formation (31). cGMP and NO have been implicated previously in the inhibition of rat parietal cell function: an NO donor induced cGMP production and inhibited H⁺ production in isolated rat parietal cells (3). However, in contrast to these findings, we have found (39) no evidence of cGMP production and specific GMP-dependent signaling systems in the present rat parietal cell system. Moreover, a cGMP analog had no effect on [¹⁴C]aminopyrine accumulation in response to histamine or carbachol but rather acted to stimulate basal H⁺ production by cross-activation of the cAMP-dependent protein kinase (39). These findings clearly argue against a role of cGMP as mediator of the antisecretory IL-1β effect observed in the present study.

The inhibitory effect of IL-1β on isolated parietal cells is in line with previous in vivo studies (32). Thus, in addition to interacting with central nervous sites, peripheral IL-1β acting at parietal cell receptors may be a paracrine antiserumatory agent. Our data indicate that the cytokine is more effective in inhibiting the parietal cell response to cholinergic neural stimulation than that to the physiologically more important humoral secretagogue histamine. Release of the latter, however, is efficiently impaired by a direct effect of IL-1β on rat fundic ECL cells (31). Thus, by interacting with specific receptors on both ECL and parietal cells, peripheral IL-1β has the potential to reduce histamine-dependent gastric acid secretion as well as the parietal cell response to cholinergic stimulation. The antisecretory effect of peripheral IL-1β may be of physiological relevance. This hypothesis is not disproved by the observation that in pylorus-ligated rats basal gastric acid secretion was unaffected by an IL-1R antagonist (35), because in this study the antagonist was applied intracerebroventricularly, compromising firm conclusions as to the role of peripheral IL-1β. On the other hand, a pathophysiological relevance of the antisecretory effect of peripheral IL-1β is supported by the general observation that the cytokine is not produced constitutively but rather in response to inflammation, endotoxins, burn injury, or sepsis (18, 27, 53). It may be speculated that under these conditions the antisecretory effect of IL-1β helps to prevent acid-induced lesions of the gastric mucosa. In line with that, increased gastric mucosal expression of IL-1β has been observed during the healing phase of experimental ulcers (54). The direct inhibitory effect of IL-1β on parietal (present study) and ECL cells (31), potentially together with effects of other inflammatory mediators and bacterial factors, may contribute to hypochlorhydria during acute infection with H. pylori (13, 24, 25, 48) when major parietal cell damage is morphologically absent. Furthermore, compared with preferential H. pylori infection of the gastric antrum mucosa, patients with intense H. pylori colonization of the gastric body are relatively
protected against duodenal ulcers (21), which are usually associated with hypersecretion of gastric acid. It may be speculated that the high local release of antisecre-

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