IL-1β modulation of H,K-ATPase α-subunit gene transcription in Helicobacter pylori infection

Arindam Saha, Charles E. Hammond, Monika Gooz, and Adam J. Smolka

Department of Medicine, Medical University of South Carolina, Charleston, South Carolina

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Saha A, Hammond CE, Gooz M, Smolka AJ. IL-1β modulation of H,K-ATPase α-subunit gene transcription in Helicobacter pylori infection. Am J Physiol Gastrointest Liver Physiol 292: G1055–G1061, 2007. First published October 1, 2006; doi:10.1152/ajpgi.00338.2006.—Helicobacter pylori infection of the human gastric body induces hypochlorhydria by perturbing acid secretion. H. pylori inhibits parietal cell H,K-ATPase α-subunit (HKα) gene and protein expression, providing a mechanistic basis for clinical hypochlorhydria. Given that H. pylori infection increases gastric mucosal IL-1β, an acid secretory inhibitor, we investigated the role of IL-1β in H. pylori-mediated inhibition of HKα transcription. Human gastric adenocarcinoma (AGS) cells were transfected with promoter-reporter constructs containing human HKα 5′-flanking sequence deletions. IL-1β (10 ng/ml) had no effect on the transcriptional activity of six progressively shorter deletion constructs of the HKα promoter (HKα2179–HKα340) and significantly stimulated the activity of HKα206, HKα177, HKα165, and HKα102 deletion constructs (80%, 100%, 46%, and 35%, respectively). H. pylori inhibited the transcriptional activity of HKα2179, HKα206, HKα177, HKα165, and IL-1β relieved the H. pylori inhibition of HKα2179 and HKα206 activity but not HKα177 and HKα165 activity. AGS cell pretreatment with a MEK1/2 inhibitor prevented the IL-1β-mediated stimulation, but p38 and JNK pathway inhibitors did not. IL-1β mRNA levels in AGS cells were low and unaffected by H. pylori, and ELISAs of H. pylori-conditioned AGS culture media showed no measurable IL-1β secretion. These data indicate that an IL-1β-dependent cis-response element lies downstream of −206 nt in the HKα promoter and that IL-1β-mediated upregulation of HKα transcription is affected by an ERK1/2 kinase signal pathway. We conclude that an IL-1β-responsive HKα cis element positively regulates HKα gene transcription in shortened deletion constructs and that H. pylori-induced inhibition of HKα transcription is not mediated by IL-1β.

interleukin-1β; gastric acid secretion

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to activate constitutive HKα transcription in canine parietal cells (23), and protein binding to a site 100 bp further upstream (homologous to the c-fos serum response element 3' half-site) has been shown to be associated with EGF-induced transcriptional activation of the HKα gene (14). Significantly, in vitro H. pylori treatment of human gastric epithelial cells transfected with a plasmid containing a 2.2-kb human HKα 5'-flanking sequence coupled to a luciferase reporter gene downregulated HKα transcriptional activity by 60% (12).

The host acid secretory status (16) and impact of H. pylori infection on acid secretion (20) are increasingly recognized as critical elements in defining the clinical outcome. The effects of IL-1β on HKα transcriptional activity in the presence and absence of H. pylori have not been described. The present study sought to determine whether H. pylori-dependent downregulation of HKα gene transcription in gastric epithelial cells is mediated by IL-1β.

MATERIALS AND METHODS

Cells, media, and reagents. Human gastric adenocarcinoma (AGS) cells (CRL1739) and H. pylori (strain ATCC 49503) were purchased from the American Type Culture Collection (Manassas, VA). Ham’s F-12 containing L-glutamine was obtained from Mediatech (Herndon, VA), and Opti-MEM was purchased from Invitrogen (Carlsbad, CA). Brucella broth and microaerophilic gas packs were from BD Biosciences ( Sparks, MD). FBS was acquired from Atlanta Biological (Norcross, GA). Phorbol 12-myristate 13-acetate (PMA) and JNK inhibitor II were from Calbiochem-Novabiochem (La Jolla, CA), and IL-1β was purchased from Sigma-Aldrich (St. Louis, MO). Restriction enzymes, MAPK inhibitors PD-98059 and SB-203580, pGL2-Basic Vector transfection plasmid, and luciferase assay substrate were obtained from Promega (Madison, WI). The transfection plasmid pMaxGFP was purchased from Amaxa (Gaithersburg, MD), and FuGene-6 transfection reagent was from Roche Diagnostics (Indianapolis, IN). The iScript cDNA synthesis kit was from Bio-Rad Laboratories (Hercules, CA). All other reagents were of molecular biology grade with maximum possible purity.

Cell and bacterial cultures. AGS cells were grown in Ham’s F-12 containing L-glutamine supplemented with 10% FBS at 37°C in a humidified incubator with 5% CO₂-95% air. AGS cells were used representing a portion of the human gastric HKα, H,K-ATPase α-subunit.

Table 1. HKα 5'-flanking sequence deletion constructs transiently transfected into gastric adenocarcinoma cells in the form of pGL2-Basic luciferase plasmids

<table>
<thead>
<tr>
<th>Deletion Construct</th>
<th>Base Pair Number Relative to the Transcriptional Start Site</th>
<th>Fragment Length, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>HKα2179</td>
<td>−2179 to + 20</td>
<td>2,199</td>
</tr>
<tr>
<td>HKα1552</td>
<td>−1552 to + 20</td>
<td>1,572</td>
</tr>
<tr>
<td>HKα1376</td>
<td>−1376 to + 20</td>
<td>1,396</td>
</tr>
<tr>
<td>HKα758</td>
<td>−758 to + 20</td>
<td>778</td>
</tr>
<tr>
<td>HKα589</td>
<td>−589 to + 20</td>
<td>609</td>
</tr>
<tr>
<td>HKα340</td>
<td>−340 to + 30</td>
<td>370</td>
</tr>
<tr>
<td>HKα206</td>
<td>−206 to + 20</td>
<td>226</td>
</tr>
<tr>
<td>HKα177</td>
<td>−177 to + 30</td>
<td>207</td>
</tr>
<tr>
<td>HKα165</td>
<td>−165 to + 30</td>
<td>195</td>
</tr>
<tr>
<td>HKα102</td>
<td>−102 to + 30</td>
<td>132</td>
</tr>
<tr>
<td>HKα64</td>
<td>−64 to + 30</td>
<td>94</td>
</tr>
<tr>
<td>HKα58</td>
<td>−58 to + 30</td>
<td>88</td>
</tr>
</tbody>
</table>

HKα, H,K-ATPase α-subunit.

DNAs were then ligated to appropriately linearized pGL2-Basic Vector plasmid using T4 DNA ligase.

Transient transfection. AGS cells (10⁵ cells/well) were cultured overnight in 24-well cell culture plates, washed with PBS, and then treated for 24 h with Opti-MEM containing 0.2 μg DNA and FuGene-6 transfection reagent at a DNA mass-to-FuGene-6 volume ratio of 1:6. AGS cells were cotransfected with pMaxGFP to provide a normalization control and a measure of transfection efficiency, and pGL2Basic plasmids containing neither the promoter nor enhancer served as negative controls. The ratio of promoter-reporter plasmids to normalization control plasmids was 1:1. After 24 h of transfection, cells were treated with 100 nM PMA in FBS-supplemented Ham’s F-12, H. pylori, and/or IL-1β for different periods of time. When needed, AGS cells were incubated with inhibitors of intracellular signaling pathways for 90 min before IL-1β treatment. Cells were lysed with 1× passive lysis buffer, and light emission was measured as relative light units (RLUs) in a Victor 1420 Multi-Label Counter (Perkin-Elmer Bio-Sciences) using the luciferase assay substrate according to the manufacturer’s protocol. Fluorescence of pMaxGFP reporter plasmids was measured at 485-nm excitation and 538-nm emission in a Spectramax Gemini EM Spectra-Fluorometer ( Molecular Devices, Sunnyvale, CA). Data from individual wells were normalized to cotransfected pMAX-GFP fluorescence and corrected by subtracting normalized promoterless pGL2-Basic Vector RLU data. Data points are shown as means ± SD of three independent transfection experiments with each deletion construct.

IL-1β ELISA. AGS cells were cultured to 75–80% confluency in T-75 flasks and harvested after trypsin-EDTA digestion for 15 min followed by the addition of Ham’s F-12 medium containing 10% FBS. Cells were centrifuged at 1,500 rpm for 5 min, resuspended in Ham’s F-12 medium with 10% FBS, and counted by hemocytometry, and cell viability was measured by trypan blue exclusion. AGS cells (250,000 cells/well) were plated in six-well cell culture plates and grown for 20 h. Cells were transferred to serum-free medium for 15–20 h and then treated for varying periods of time with 100 nM PMA and/or H. pylori at a MOI of 25. The IL-1β content of aliquots of cell culture supernatant was measured by ELISA using an IL-1β Duo Set ELISA Development System (R&D Systems, Minneapolis, MN) according to the manufacturer’s protocol.

Real-time RT-PCR. AGS cells were grown to 75–80% confluency in 24-well cell culture plates, and culture was continued in the absence of FBS for 15–20 h. H. pylori bacteria were then added to the cells at a MOI of 25 for varying periods of time (0–12 h). AGS cell RNA was isolated using RNA STAT-60 reagent from Tel Test (Friendswood, TX) and reverse transcribed using a iScript cDNA synthesis kit according to the manufacturer’s protocol. Measurements of AGS cell
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IL-1β mRNA were carried out by real-time RT-PCR using an iCycler iQ with iQ SYBR Green Super mix (Bio-Rad, Hercules, CA) and forward (5′-CAGGATGACCTGTACGATCA-3′) and reverse (5′-TGGAGCTTTCGTT-3′) primers specific for human IL-1β.

Statistical analysis. Data acquired from experiments with multiple samples subjected to each treatment were analyzed by two-way ANOVA. Levels of significance in multiple pairwise comparisons of treatment and control groups were calculated using Bonferroni post test analysis as implemented in the GraphPad PRISM version 4 statistical software package. Data are expressed as means ± pooled SD, and statistical significance was ascribed to $P$ values of <0.05.

RESULTS

Regulation by H. pylori of the transcriptional activity of the HKα 5′-flanking region transfected into cultured human AGS cells has been reported previously (12), and this model system was used in the present study to investigate the effect of exogenously administered IL-1β on HKα promoter activity. To test the hypothesis that HKα promoter inhibition might be mediated by IL-1β, AGS cells were transiently transfected with a series of HKα deletion constructs (Table 1), and maximal transcriptional activity was induced by 100 nM PMA. Cells were then treated with 10 ng/ml IL-1β, and HKα promoter activity was measured after 24 h. As shown in Fig. 1, constitutive activities of deletion constructs from HKα2179 through HKα340 remained relatively constant ($P > 0.05$) and were unaffected by IL-1β ($P > 0.05$). In the absence of IL-1β, constitutive activities of deletion constructs HKα206 through HKα102 increased up to twofold, whereas those of the shortest constructs (HKα64 and HKα58) were significantly attenuated. In the presence of IL-1β, activities of HKα206, HKα177, HKα165, and HKα102 deletion constructs were significantly increased compared with untreated controls ($P < 0.001$). Activities of HKα206 and HKα177 constructs were stimulated ~2-fold and those of HKα165 and HKα102 constructs were stimulated by 1.4-fold relative to constitutive levels.

Transcriptional activities of HKα177 and HKα165 deletion constructs were regulated by IL-1β in a dose-dependent manner, as shown in Fig. 2. At all doses of IL-1β, promoter activities of HKα177 and HKα165 were significantly increased compared with untreated control activities ($P < 0.05$). HKα177 promoter activities with 10 and 100 ng/ml IL-1β did not differ but were significantly increased compared with 1 ng/ml IL-1β ($P < 0.05$). HKα165 promoter activities did not differ significantly from one another at any measured concentration of IL-1β ($P > 0.05$). These data indicated that a repressor-binding sequence is located between −340 bp and −206 bp in the HKα 5′-flanking region and that IL-1β-responsive cis-activation elements are located between −206 and −64 bp.

To further characterize the sensitivity of HKα177 and HKα165 deletion constructs to IL-1β, the time course of IL-1β response was determined by real-time RT-PCR (Fig. 3). In the absence of IL-1β, activities of HKα206 through HKα102 constructs were regulated by IL-1β in a dose-dependent manner, as shown in Fig. 4.
stimulation of both constructs was examined. As shown in Fig. 3, time-dependent HKα177 and HKα165 promoter activities as a function of IL-1β treatment were not significantly different at 0.5, 1, and 4 h ($P > 0.05$) but were significantly different at 8, 16, and 24 h ($P < 0.001$). In the absence of IL-1β, transcriptional activities of both deletion constructs were maximally stimulated at 16 and 24 h. HKα177 activity at both time points was increased by approximately twofold after treatment of transfected cells with IL-1β (10 ng/ml; shaded bars) or without IL-1β (open bars) for varying periods of time.

Because these results clearly argued against an IL-1β-mediated mechanism of *H. pylori* inhibition of HKα gene transcription, we next investigated the effects of *H. pylori* on both of the HKα deletion constructs maximally activated by IL-1β (HKα177 and HKα165). AGS cells were transiently transfected with HKα177 and HKα165 deletion constructs and then incubated for 24 h with differing concentrations of *H. pylori*. Transcriptional activities of these two HKα sequences containing putative IL-1β cis-response elements were not significantly inhibited by *H. pylori* at a MOI of 10 ($P > 0.05$) but were significantly inhibited by *H. pylori* at MOIs of 25 ($P < 0.01$) and 50 ($P < 0.001$) compared with untreated controls. For both constructs, activity differences between *H. pylori* MOIs of 10 and 25 and between MOIs of 25 and 50 were insignificant ($P > 0.05$); however, differences between *H. pylori* MOIs of 10 and 50 were significant ($P < 0.001$). Although maximal inhibition (63%) of both constructs was induced at a *H. pylori* MOI of 50 (Fig. 4), at this MOI AGS cells showed significant deterioration of normal morphology, with pronounced vacuolization and blebbing, and so *H. pylori* MOIs were adjusted to 25 in subsequent experiments.

Given the observed stimulation of HKα177 and HKα165 transcriptional activity by IL-1β, we next studied the effects of IL-1β on the transcriptional activity of HKα 5′-deletion constructs in the setting of *H. pylori* infection of transfected AGS cells. HKα2179 (full length), HKα206, HKα177, and HKα165 deletion constructs were transiently transfected into AGS cells and incubated for 24 h with 100 nM PMA and *H. pylori* at a MOI of 25 with and without 10 ng/ml IL-1β. Activities of all four deletion constructs were significantly inhibited by *H. pylori* (Fig. 5). Measurements of normalized luciferase RLUs showed that treatment of transfected cells with *H. pylori* inhibited the promoter activity of HKα2179 and HKα206 by 27% and 67%, respectively, and that this inhibition did not occur in the presence of IL-1β. In contrast, although *H. pylori* inhibited HKα177 and HKα165 by 37% and 31%, respectively, this inhibition was not significantly relieved by IL-1β (Fig. 5). These data suggest that activation of an IL-1β-sensitive cis-response element located between −206 and −177 bp relieves *H. pylori*-mediated regulatory effects on the HKα 5′-flanking region.

Given the presence of IL-1β-responsive sequence elements in the HKα promoter and their modulatory role in *H. pylori*-mediated HKα inhibition, we investigated the intracellular signaling pathways through which IL-1β regulates HKα gene expression. AGS cells were transiently transfected with HKα2179, HKα177, HKα165, or HKα102 deletion constructs and then incubated with 10 ng/ml IL-1β and with one of three inhibitors of specific MAPK signaling pathways: PD-98059, an ERK1/2 pathway inhibitor (50 μM); SB-203580, a p38 pathway inhibitor (10 μM); and JNK inhibitor II, an inhibitor of the JNK pathway (100 nM). As shown in Fig. 6, activities of all four deletion constructs were significantly inhibited in the presence of the ERK1/2 inhibitor (PD-98059) compared with untreated control activities or activities in the presence of IL-1β ($P < 0.001$). In the cases of the p38 and JNK pathway inhibitors, there were no significant differences in activities compared with untreated control activities or activities in the presence of IL-1β. These data indicate that HKα promoter

![Fig. 3. Time course of IL-1β stimulation of the transcriptional activation of HKα177 and HKα165 deletion constructs. AGS cells were transiently transfected with HKα177-Luc (A) or HKα165-Luc (B) and then incubated with 100 nM PMA with IL-1β (10 ng/ml; shaded bars) or without IL-1β (open bars) for varying periods of time.](http://ajpgi.physiology.org/)

![Fig. 4. Effect of *Helicobacter pylori* on the transcriptional activity of HKα177 and HKα165 deletion constructs. AGS cells were transiently transfected with HKα177-Luc (■ and thick solid line) or HKα165-Luc (○ and thin solid line) and treated with increasing multiplicities of infection (MOIs) of *H. pylori*.](http://ajpgi.physiology.org/)
activity in the targeted deletion constructs is dependent on a functional ERK1/2 signaling pathway and IL-1β stimulation of HKα promoter activity is mediated through the same pathway; neither the p38 nor JNK signaling pathways are involved in the regulation of HKα gene expression.

At the outset of this study, we had sought to determine whether infection of AGS cells by H. pylori itself induced cellular production and secretion of IL-1β comparable with the epithelial secretion of IL-8 induced by H. pylori. AGS cells were infected for varying periods of time with H. pylori at a MOI of 25, and the cellular content of IL-1β mRNA was measured by quantitative RT-PCR and the IL-1β concentration of cell culture media was measured by ELISA. IL-1β mRNA levels in control AGS cells were very low and unchanged following H. pylori infection (data not shown). IL-1β was undetectable by ELISA in the culture media of control or H. pylori-infected cells (data not shown). These results suggest that our observation of IL-1β effects on HKα gene expression reflect exogenous, i.e., leukocyte and phagocyte, sources of IL-1β in the physiological setting rather than local autocrine effects.

DISCUSSION

We (12) previously reported that in vitro H. pylori infection of cultured gastric epithelial cells transfected with HKα promoter-reporter constructs resulted in the significant downregulation of HKα transcriptional activity. In that study, AGS cells were shown to possess functional histamine and EGF receptors whose activation modulated the transcriptional activity of transfected plasmids incorporating a 2.2-kb human HKα 5′-flanking sequence fused to a luciferase reporter gene. H. pylori infection of transfected AGS cells dose dependently inhibited basal and histamine-stimulated HKα promoter activities, and dose dependently inhibited PMA-induced and staurosporine- and calphostin C-sensitive activation of the HKα promoter. Also, H. pylori dose dependently inhibited EGF activation of HKα promoter activity. These data suggested that H. pylori infection downregulates HKα gene expression via intracellular pathways involving protein kinase C and protein tyrosine kinase (12). The possibility that HKα gene expression was modulated in the transfected AGS cell model by IL-1β, originating in H. pylori-infected cells and acting in an autocrine capacity, was not addressed in that study.

Mucosal levels of the proinflammatory cytokine IL-1β are increased following gastric colonization by H. pylori, and IL-1β is known to be a potent inhibitor of acid secretion. In the present study, we sought to determine whether IL-1β activates signaling pathways in gastric epithelial cells that ultimately downregulate HKα transcription. Such a downregulation would lower the expression of functional proton pumps and...
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Our demonstration that an intact functional ERK1/2 signaling pathway is necessary to elicit IL-1β stimulation of HKα deletion construct activity (Fig. 6) complements and extends the spectrum of AGS cell signaling pathways induced by IL-1β receptor activation. Thus, IL-1β has been shown to dose-dependently increase IL-8 secretion by AGS cells, and this effect was significantly reduced by the protein tyrosine kinase inhibitors herbimycin A and genistein; protein kinase C inhibition did not reduce IL-1β stimulation of IL-8 secretion (3). The transcription factor mobilized by IL-1β and H. pylori to upregulate IL-8 gene expression is known to be NF-κB (33). Also, IL-1β has been shown to dose-dependently enhance AGS cell proliferation, and this effect was blocked by an IL-1 receptor antagonist (1). IL-1β-stimulated proliferation was blocked by genistein and by a specific inhibitor (PD-98059) of the ERK1/2 pathway (1). On the other hand, in the case of IL-1β dose-dependent stimulation of cyclooxygenase-2 mRNA and protein expression levels and prostaglandin E2 production and secretion in AGS cells, activation of both the ERK1/2 and p38 pathways was shown to be required (8). The involvement of the JNK signaling pathway in IL-1β-mediated events in AGS cells has not been reported.

The concentration at which IL-1β exerted regulatory effects on the HKα promoter in this study is the same (10 ng/ml) as that reported to dose-dependently inhibit basal and stimulated acid secretion in isolated rabbit parietal cells (2) and to stimulate gastrin release from rabbit antral G cells (39). Comparable IL-1β concentrations (25 ng/ml) were reported to stimulate maximal IL-8 release by AGS cells (15). In contrast, in isolated rat parietal cells, acid secretion stimulated by histamine or carbachol was maximally inhibited (by 35% and 67%, respectively) by the preincubation of cells with 2.5 pg/ml IL-1β (32). These differences in IL-1β potency with respect to acid secretion regulation may reflect species- and/or cell-specific differences in signaling mechanisms regulating acid secretion that are impacted by IL-1β receptor activation. Thus, in rat parietal cells, IL-1β treatment has been shown to impair an inositol (1,4,5)-trisphosphate-dependent increase in intracellular Ca2+ concentration and had no significant effect on histamine-stimulated cAMP production (32), whereas, in rabbit parietal cells, IL-1β inhibited forskolin- but not dibutyryl-cAMP-stimulated acid secretion (2), implicating IL-1β impairment of cAMP generation but no impairment of protein kinase A activity or protein kinase A-mediated downstream effectors.

Although the mechanism by which IL-1β inhibits acid secretion at the level of parietal cells remains to be clarified, the present study excludes direct IL-1β-mediated perturbation of routine transcription factor interactions with the HKα promoter as a potential mechanism, at least in the AGS cell model investigated here. We can conclude, however, that 1) an IL-1β-dependent cis-activation sequence is located between −206 and −102 bp in the HKα promoter and that repressor sequences are located between −206 and −340 bp; 2) IL-1β modulates H. pylori-mediated HKα promoter inhibition; and 3) IL-1β exerts HKα promoter effects through MEK1/2 MAPK signal transduction pathways.

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REFERENCES


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