Oxidative Stress Activates the Human Histidine Decarboxylase Promoter in AGS Gastric Cancer Cells

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Oxidant stress is thought to play a role in the pathogenesis of many gastric disorders. We have recently reported that histidine decarboxylase (HDC) promoter activity is stimulated by gastrin through a protein kinase C- and extracellular signal-regulating kinase (ERK)-dependent pathway in gastric cancer (AGS-B) cells, and this transcriptional response is mediated by a downstream cis-acting element, the gastrin response element (GAS-RE). To study the mechanism through which oxidant stress affects gastric cells, we examined the effects of hydrogen peroxide (H2O2) on HDC promoter activity and intracellular signaling in AGS-B cells. H2O2 (10 mM) specifically activated the HDC promoter 10–12-fold, and this activation was blocked by both mannosyl and N-acetylcysteine. Hydrogen peroxide treatment of AGS-B cells increased the phosphorylation and kinase activity of ERK-1 and ERK-2, but did not affect Jun kinase tyrosine phosphorylation or kinase activity. In addition, treatment of AGS-B cells with H2O2 resulted in increased c-fos::c-jun mRNA expression and AP-1 activity, and also led to increased phosphorylation of epidermal growth factor receptor (EGFR) and Shc. H2O2-dependent stimulation of HDC promoter activity was completely inhibited by kinase-deficient ERks, dominant-negative (N17 and N15) Ras, and dominant-negative Raf, and partially blocked by a dominant-negative EGFR mutant. In contrast, protein kinase C blockade did not inhibit H2O2-dependent induction of the HDC promoter. Finally, deletion analysis demonstrated that the H2O2 response element could be mapped to the GAS-RE (nucleotides 2 to 24) of the basal HDC promoter. Overall, these studies suggest that oxidant stress activates the HDC promoter through the GAS-RE, and through an Ras-, Raf-, and ERK-dependent pathway at least partially involving the EGFR.

Reactive oxygen metabolites (ROMs),1 such as superoxide anion O2−, hydroxyl radical OH, and H2O2, are generated in cells as physiological by-products of electron transfer reactions and arachidonic acid metabolism (1, 2). An elevated level of ROMs reflecting oxidative stress is observed during a number of acute physiologic and pathological states, including sepsis, exposure to ionizing radiation, ischemia and reperfusion, and diverse inflammatory conditions (2). In addition, oxidative stress is thought to play a role in many chronic disease processes, included atherosclerosis, aging, and cancer (3–5).

The gastric mucosa is continuously exposed to luminal oxidants generated from ingested food, bacteria, and shed mucosal cells (6). The gastric epithelium, in conjunction with the surface mucous layer, represents the first line of defense against luminal oxidative stress (7); despite constant exposure to luminal oxidants, the gastric epithelium remains unaffected. However, enhanced production of ROMs due to acute and chronic inflammation of the gastrointestinal tract may contribute to the mucosal injury. Inflammatory and ulcerative diseases of the gastric mucosa, such as those associated with ethanol, nonsteroidal anti-inflammatory drugs, cold stress, burn stress, ischemia-reperfusion, and Helicobacter pylori, are associated with increased oxidative stress (8–10). H. pylori-infected gastric mucosa, for example, is characterized by an accumulation of active neutrophils (polymorphonuclear leukocytes) (11, 12), and these activated neutrophils may result in increased oxidative DNA damage (13). Thus, reactive oxygen metabolites may be involved in the pathogenesis of both peptic ulcer disease and gastric cancer (14).

Although oxidative stress has cytotoxic effects, studies have also indicated that ROMs mediate a number of adaptive biologic responses (15). Oxidative stress secondary to H2O2 exposure may function as a local trigger for programmed cell death (16), but it can also modulate the expression of a variety of genes that are involved in the immune and inflammatory response, such as the nuclear transcription factor kB (NF-kB) (17). In addition, ROMs at low levels have been shown to induce the expression of growth factor-regulated genes, such as AP-1 (c-fos and c-jun), c-myc, and egr-1 (17–19). Oxidative stress appears to affect numerous signaling pathways through protein phosphorylation and induce selectively a number of genes, and thus chronic exposure to low levels of ROM may conceivably influence cell growth and differentiation.

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1 The abbreviations use are: ROM, reactive oxygen metabolites; HDC, histidine decarboxylase; hHDC, human HDC; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; JNK, Jun kinase; GAS-RE, gastrin response element; PKC, protein kinase C; Luc, luciferase; CAT, catecholamine acetyltransferase; PMA, phorbol 12-myristate 13-acetate; MAP, mitogen-activated protein; DN, dominant-negative; AP-1, activator protein 1; kb, kilobase pair(s).
Histidine decarboxylase (HDC) is the major enzyme involved in the production of histamine from the amino acid L-histidine, a key step in the stimulation of gastric acid secretion (20). Both histamine and HDC enzyme are activated in the rat stomach in response to cold-induced stress (21, 22). Several previous observations have suggested that the HDC gene may be activated in the stomach under conditions of oxidative stress. For example, HDC activity has been shown to be increased during ischemia-reperfusion injury to the gastrointestinal tract (23–25). Experiments from our laboratory have shown that the HDC promoter is transcriptionally regulated by gastrin and the phorbol ester (phorbol 12-myristate 13-acetate, PMA) through a protein kinase C (PKC)- and MAP kinase-dependent pathway, acting on a 23-nucleotide cis-acting element, the gastrin response element (GAS-RE) (20, 26, 27). More recently, we have shown that activation of AP-1 is essential for gastrin-stimulated HDC transcription, although the mechanism is most likely indirect (28), indicating that HDC may be an important downstream target of AP-1. Thus, we decided to study the effects of oxidative stress on gastric epithelial cells, AP-1 activity, and HDC promoter activity. These studies suggest that H₂O₂ can selectively stimulate HDC promoter activity, most likely through an EGFR-dependent signaling pathway that leads to activation of the MAP kinase/ERK pathway.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dubbelco’s modified Eagle’s medium, fetal calf serum, penicillin-streptomycin, and EDTA-trypsin were obtained from Bio Whittaker, Walkerville, MA. Sulfated gastrin-17 was purchased from Peninsula Laboratories, San Diego, CA. The protein kinase inhibitor 1-(5-isouquinolinesulfonyl)-2-methyl-piperazine (H-7) and the phorbol ester PMA were obtained from BIOMOL Research Laboratories, Plymouth Meeting, PA. H₂O₂, N-acetylcysteine, mannotol, and dibutryl cAMP were obtained from Sigma.

**DNA Constructs and Reporter Plasmids**—The human 1.8-kb HDC-luciferase construct and the GAS-RE/thymidine kinase-Luc construct have been described elsewhere (26). The GAS-RE/thymidine kinase-Luc construct is derived from thymidine kinase-Luc but also contains the human HDC GAS-RE sequences ligated upstream of the herpex simplex virus 1 thymidine kinase promoter. The 4XTRE-CAT construct has been previously reported (28). The c-Fos-luciferase construct was a kind gift of Richard Treisman (29). The EGFR dominant-negative expression (HER653) construct was a gift of Murray Kors and contains the kinase-deleted mutant of the human EGFR cDNA (dominant-negative (DN)-EGFR) under the control of the cytomegalovirus (CMV) promoter. Transfection of this DN-EGFR construct has been shown to block EGFR signaling (30). The ornithine decarboxylase-luciferase construct was a gift of Jean-L. Marchand, and contained 450 base pairs of human ornithine decarboxylase 5′-flanking DNA. The GAL4-c-Jun wild type construct contains the GAL4 DNA binding domain linked to amino acids 1–246 of human c-Jun, while the GAL4-c-Jun mutant (AA) has Ser-63 and Ser-73 of c-Jun mutated to alanines. These constructs and the SXGAL-Luc system have been described previously (27).

The ERK cDNAs and mutants were subcloned into the expression vector pCMV5 (in which cDNA expression is driven by a CMV promoter) and have been described previously (27, 31). Wild type ERK constructs contained full-length human cDNAs, whereas the mutants K71R-Ala15 (RasN15) and have been described previously (28). The c-Fos-luciferase construct was a gift of Juanita L. Merchant, and contained 450 base pairs of human 5′-flanking DNA. The GAL4-c-Jun wild type expression constructs which have previously been reported (28) and have been described elsewhere (26). The GAS-RE/thymidine kinase-Luc construct and the GAS-RE/thymidine kinase-Luc construct were hybridized using Rapid-Hyb buffer and Shc (Transduction Laboratories) were used to immunoprecipitate these proteins from cells lysates. Immune complexes were collected on Protein A-Sepharose beads and washed three times with ice-cold lysis buffer. SDS-sample buffer was added, and the beads were boiled for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore). The membrane was then probed with anti-phosphotyrosine antibody (4G10) (1 μg/ml) (Upstate Biotechnology Inc.). Bands were visualized with the enhanced chemiluminescence (ECL) prime kit (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Membranes were prehybridized for 30 min and hybridized for 60 min using Rapid-Hyb buffer (Amersham Pharmacia Biotech) at 65 °C. Following hybridization the membranes were washed twice for 20 min each in 2× SSC, 0.1% SDS at 25 °C, then once in 2× SSC, 0.1% SDS at 55 °C for 20 min. Membranes were exposed to x-ray film for 16 h at −80 °C.

**Immunoprecipitation and Western Blotting**—AGS-B cells were grown to subconfluence and then starved for 36 h in Dubbelco’s modified Eagle’s medium without serum. Cells were then stimulated with H₂O₂ (10 mM final concentration) for 10 min at 37 °C. After stimulation, cells were washed in ice-cold phosphate-buffered saline and lysed in lysis buffer (20 mM Tris, pH 7.8, 150 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40, 10 mM NaF, 10 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml of each of leupeptin, pepstatin, and aprotinin). The lysates were precleared for 1 h by incubating them with normal rabbit serum and protein A-Sepharose beads (Pharmacia). Antibodies to ERK-1, JNK-1, EGF-R, and Grb2 (Santa Cruz Biotechnologies) and She (Transduction Laboratories) were used to immunoprecipitate these proteins from cells lysates. Immunocomplexes were collected on Protein A-Sepharose beads and washed three times with ice-cold lysis buffer. SDS-sample buffer was added, and the beads were boiled for 5 min. Proteins were separated by SDS-polyacrylamide gel electrophoresis on 7.5% polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore). The membrane was then probed with anti-phosphotyrosine antibody (4G10) (1 μg/ml) (Upstate Biotechnology Inc.). Bands were visualized with the enhanced chemiluminescence (ECL) prime kit (Amersham Pharmacia Biotech). Membranes that were probed with anti-phosphotyrosine antibody were reprobed after stripping the membrane. Stripping was performed by washing extensively with TTBS (20 mM Tris pH 7.6, 150 mM NaCl plus 0.05% Tween 20) for 4 h, then incubating the membrane in a solution of 62.5 mM Tris-HCl, pH 6.8, 2% SDS, and 100 mM 2-mercaptoethanol for 30 min at 55 °C followed by washing in TTBS for 2 h changing the buffer every 30 min.

**MAP Kinase Activity Assays**—To determine ERK activity in response to H₂O₂, cell-free extracts were prepared after treatment of subconfluent AGS-B cells with H₂O₂ (10 mM). Extracts were boiled with Laemmli sample buffer, and aliquots (10 μg protein) were separated on a 10% SDS-polyacrylamide gel containing 0.5 mg/ml ERK substrate myelin basic protein (35). Gels were subsequently submitted to denaturation-renaturation and kinase activity was measured by incubating the gels in kinase buffer containing 25 μM ATP and 100 μM [γ-32P]ATP for 90 min at room temperature. Kinase reaction was terminated by washing the gels in 5% trichloroacetic acid, 10 mM sodium pyrophosphate. Dried gels were exposed to x-ray films, and signal intensity was determined by scanning densitometry using a Molecular Dynamics personal densitometer with Imagequant version 3.22 software.

**RESULTS**

**Hydrogen Peroxide Activates the HDC Promoter**—To determine whether oxidative stress stimulates HDC promoter activity, AGS-B cells stably transfected with the 1.8-kb hHDC-Luc construct were exposed to varying concentrations of hydrogen peroxide precipitation technique (DNA transfection kit, 5 Prime Inc.) as described previously (20). H₂O₂ stimulation was performed for 10 min, and cells were harvested 6 h thereafter. In time course studies, cells were harvested 0, 2, 4, and 6 h after a 10-min H₂O₂ stimulation. Gastrin and PMA were added at stimulatory concentrations (12–24 μM) after transient transfection. Cells were harvested, and luciferase assays were performed at 48 h. Luciferase assays were carried out using luciferin, ATP, coenzyme A (Promega system) with a Monolight Luminometer (Analytical Luminescence Laboratory). CAT assays were performed as described previously (28). Experiments were performed in triplicate or quadruplicate, and results were calculated as mean ± S.D. You-nam et al. (36) have shown that activation of AP-1 is essential for gastrin-stimulated HDC transcription, although the mechanism is most likely indirect (28), indicating that HDC may be an important downstream target of AP-1. Thus, we decided to study the effects of oxidative stress on gastric epithelial cells, AP-1 activity, and HDC promoter activity. These studies suggest that H₂O₂ can selectively stimulate HDC promoter activity, most likely through an EGFR-dependent signaling pathway that leads to activation of the MAP kinase/ERK pathway.

**Oxidative Stress Activates Human Histidine Decarboxylase**

23407
H2O2 for 10 min. HDC promoter activity in response to various concentrations of H2O2 (0–10 mM) showed an increase in activity compared with untreated controls and represents the mean ± S.E. of four separate experiments and is expressed as the fold increase over untreated controls. The inhibitors alone had no effect on HDC-Luc activity.

**Hydrogen Peroxide Activates AP-1 and c-Myc in AGS-B Gastric Cells**—Previous studies from our laboratory showed that HDC promoter activity was stimulated by gastrin and PMA through an AP-1-dependent mechanism (28). Therefore, we examined the effect of H2O2 on the expression of c-fos and c-jun mRNA in AGS-B gastric cells using Northern blot analysis. Treatment of AGS-B cells with 10 mM H2O2 led to a marked increase in mRNA expression of both c-fos and c-jun, with initial increases seen at 30 min and the largest increases observed at 120 min (Fig. 3A). We also examined the effect of H2O2 on c-Jun-dependent transactivation, using the GAL4-c-Jun (wild type and mutant) expression vectors and the 5XGAL-Luc reporter gene (see “Experimental Procedures”). Both H2O2 and PMA stimulated transactivation by GAL4-c-Jun wild type but had no effect on the mutant construct, GAL4-c-Jun mutant (AA) (Fig. 3B). Hydrogen peroxide also stimulated AP-1-dependent transactivation, as measured using AGS-B cells transiently transfected with the 4XTRE-CAT construct (Fig. 3C). The effect of H2O2 on c-fos gene expression was most likely due to transcriptional induction, as evidenced by a 10-fold stimulation effect of H2O2 on c-fos promoter activity in transiently transfected AGS-B cells (Fig. 3D). Finally, H2O2 also stimulated c-Myc-dependent transactivation, as shown in studies using wild type and mutant GAL4-c-Myc expression vectors and the 5XGAL-Luc reporter gene (Fig. 3E). These studies indicate that H2O2 transactivates c-Myc, and that the AP-1 pathway is activated both transcriptionally and post-translationally by oxidative stress in gastric cells.

**Fig. 2**. Induction of HDC promoter activity by H2O2 is blocked by N-acetylcysteine and mannitol. AGS-B cells were transiently transfected with 1.8-kb hHDC-Luc. N-Acetylcysteine (20 mM) or mannitol (100 mM) was added where indicated 45 min before the addition of H2O2 (10 mM, where indicated) for 10 min. Cells were harvested and assayed for luciferase activity 6 h later. Luciferase activity represents the mean ± S.E. of four separate experiments and is expressed as the fold increase over untreated controls. The inhibitors alone had no effect on HDC-Luc activity.

**Fig. 1**. H2O2 stimulates the HDC promoter in AGS-B cells. Cells stably transfected with the 1.8-kb hHDC-Luc construct (28) were treated with H2O2 as indicated. Luciferase activity is expressed as increase in activity compared with untreated controls and represents means ± S.E. from four separate transfections. A, dose response curve showing HDC promoter activity in response to various concentrations (0–10 mM) of H2O2 for 10 min. B, time course showing rate of increase of HDC promoter activity in response to 10 mM H2O2. Cells were exposed to H2O2 for 10 min and then harvested and assayed for luciferase activity after the indicated time periods.
H2O2 Treatment Leads to Shc and EGFR Phosphorylation—Previous studies have suggested that in some cell systems H2O2 activates the ERKs through a Ras pathway that involves tyrosine phosphorylation of EGFR, followed by phosphorylation of Shc and the induction of Shc-Grb2-SOS complexes (3, 36). To investigate the pathways utilized by gastric cells in response to oxidant stress, we analyzed protein extracts from H2O2-treated AGS-B cells for phosphotyrosine proteins by Western blotting. H2O2 induced a marked increase in tyrosine phosphorylation of multiple proteins (Fig. 5A, lane 4) with molecular masses that ranged from 60 to 200 kDa. In order to examine the effect of H2O2 on the phosphorylation of Shc and its association with the adaptor protein Grb2, Grb2 and Shc proteins were first immunoprecipitated with specific antibodies, and phosphorylation levels were assessed using an antiphosphotyrosine antibody. As shown in Fig. 5A (lanes 5–12), treatment of AGS-B cells with H2O2 resulted in an increase in tyrosine phosphorylation of both the 52- and 46-kDa isoforms of Shc. The increase was greater than that seen with PMA or gastrin stimulation. In addition, multiple bands representing tyrosine phosphorylated proteins, not seen with PMA or gastrin stimulation, were co-immunoprecipitated when using antibodies to Grb2 or Shc (Fig. 5A).

Effect of PKC Blockade on H2O2-stimulated hHDC Promoter
Activity—To define the functional role of PKCs for H$_2$O$_2$-dependent hHDC transactivation, we down-regulated PKCs using pretreatment with 10$^{-2}$ M phorbol ester PMA as described previously (20, 26). These studies revealed that blockade of PKCs using PMA pretreatment had no effect on the transactivating effect of H$_2$O$_2$, whereas PMA-stimulated activity of the 1.8-kb hHDC-Luc construct was completely abolished (Fig. 6). These data strongly suggest that the effect of H$_2$O$_2$ on the does not stimulate JNK phosphorylation. AGS-B cells were stimulated with UV light (1 min), gastrin, PMA, H$_2$O$_2$, or left untreated (control).

**FIG. 4.** H$_2$O$_2$ stimulates ERK phosphorylation and kinase activity, but not JNK. **Top,** scanning densitometry of the MBP kinase assay blot (middle), showing activation of ERK activity by H$_2$O$_2$, gastrin, PMA, and dibutyryl (db) cAMP. **Middle,** upper panel, induction of ERK tyrosine phosphorylation by H$_2$O$_2$, gastrin, and PMA. AGS-B cells were treated for 5 min with gastrin (10$^{-7}$ m), PMA (10$^{-7}$ m), dibutyryl cAMP (10$^{-7}$ m), H$_2$O$_2$ (10 mM), or left untreated (control). Cell lysates were immunoprecipitated with anti-ERK-1 antibody, and then subjected to immunoblot analysis by anti-phosphotyrosine antibody. **Lower panel,** myelin basic protein kinase assay. AGS-B cells were stimulated with H$_2$O$_2$, gastrin, or PMA as above, or left untreated (control). To determine ERK activity in response to H$_2$O$_2$, cell-free extracts were prepared after treatment of subconfluent AGS-B cells with H$_2$O$_2$ (10 mM). Aliquots (10 $\mu$g of protein) of extracts were separated on a 10% SDS-polyacrylamide gel containing 0.5 mg/ml ERK substrate myelin basic protein. Gels were submitted to denaturation-renaturation and subsequently incubated in kinase buffer containing [$\gamma$-32P]ATP. After termination of kinase reaction, dried gels were exposed to x-ray films, and signal intensity was determined by scanning densitometry (Molecular Dynamics densitometer, Imagequant 3.22 software). **Bottom,** H$_2$O$_2$ does not stimulate JNK phosphorylation. AGS-B cells were stimulated with UV light (1 min), gastrin, PMA, H$_2$O$_2$, or left untreated (control). 

**FIG. 5.** H$_2$O$_2$ stimulates tyrosine phosphorylation of multiple proteins. A, H$_2$O$_2$ stimulates tyrosine phosphorylation of Shc and its association with Grb2. Lanes 1–4 show a Western blot of AGS-B cell lysates probed with the anti-phosphotyrosine antibody. Prior to lysis, AGS-B cells were stimulated for 5 min with either gastrin (10$^{-7}$ m), PMA (10$^{-7}$ m), H$_2$O$_2$ (10 mM), or left untreated (control). Lanes 5–12 show a Western blot (probed with anti-phosphotyrosine antibody) of the same lysates that had been first immunoprecipitated with antibodies to either Grb2 or Shc. Both the 52- and 46-kDa forms of phosphorylated Shc are apparent in the gastrin and PMA lanes (predominantly 52 kDa). Additional bands are present in the H$_2$O$_2$ lanes. B, H$_2$O$_2$ stimulates tyrosine phosphorylation of the EGFR. AGS-B cells were unstimulated (control) or stimulated with either PMA or H$_2$O$_2$. After immunoprecipitation with antibodies to Shc or EGFR, the immunoprecipitates were run on SDS-polyacrylamide gel electrophoresis, 10% gel, and transferred to polyvinylidene difluoride membrane. The blot was then probed with anti-phosphotyrosine antibody. The arrows show either the Shc band (predominantly 52 kDa) or EGF-R (170-kDa band) with H$_2$O$_2$. 

**Activity**—To define the functional role of PKCs for H$_2$O$_2$-dependent hHDC transactivation, we down-regulated PKCs using pretreatment with 10$^{-8}$ M phorbol ester PMA as described previously (20, 26). These studies revealed that blockade of PKCs using PMA pretreatment had no effect on the transactivating effect of H$_2$O$_2$, whereas PMA-stimulated activity of the 1.8-kb hHDC-Luc construct was completely abolished (Fig. 6). These data strongly suggest that the effect of H$_2$O$_2$ on the
The effect of H2O2 on the hHDC promoter activity was studied. AGS-B cells transfected with the 1.8-kb hHDC-Luc construct were pretreated with the phorbol ester PMA (10 nM for 24 h) and subsequently stimulated with a maximally effective concentration of H2O2 (10 mM) for 6 h. Luciferase activity is expressed as a fold increase relative to control transfectants and represents the mean ± of four separate experiments. The asterisk (*) indicates a statistically significant difference (p < 0.05) compared with no PMA-PT.

hHDC promoter is preferentially mediated through activation of pathways independent of PKCs.

The Effect of H2O2 on the hHDC Promoter Is Mediated through MAP Kinase/ERK Signaling Pathways—To evaluate the importance of ERK-dependent signaling pathways for H2O2-dependent regulation of the hHDC promoter, AGS-B cells were co-transfected with 1.8-kb hHDC-Luc, and expression constructs encoding dominant-negative ERK mutants and subsequently stimulated with a maximal concentration of H2O2. Expression of dominant-negative ERK proteins abolished the effect of H2O2 on hHDC promoter activity (Fig. 7), demonstrating that H2O2-dependent transactivation of the hHDC promoter is mediated through activation of MAP kinase/ERKs.

The Effect of H2O2 on the hHDC Promoter Is Mediated through Ras- and Raf-mediated Pathways—To define the initial steps in activation of intracellular signaling cascades transmitting the effect of H2O2 on the hHDC promoter, we analyzed the role of Ras and Raf for H2O2-stimulated hHDC promoter activity. Expression of the interfering Ras mutants Ha-ras-ASN17 (RasN17) or Ha-ras-Ala15 (RasN15) abolished the effect of H2O2 on the hHDC construct (Fig. 8). In contrast, no effect on PMA-stimulated hHDC promoter transactivation could be observed (27) (data not shown). Similarly, inhibition of Raf by expression of a dominant-negative Raf-1 mutant abolished the H2O2 effect (Fig. 8). Raf blockade also inhibited hHDC promoter transactivation in response to PMA (27) (data not shown). Taken together, our data demonstrate transduction of H2O2-dependent hHDC transactivation through a Ras- and Raf-dependent proximal pathway.

H2O2 Induction of the HDC Promoter Is Partially Blocked by Dominant-Negative EGFR—The role of EGFR in mediating stimulation of the HDC promoter was further studied using a kinase-deficient EGFR mutant lacking the cytoplasmic domain, which inhibits EGFR downstream signaling by formation of signaling-defective heterodimers with the wild-type receptor. This DN-EGFR construct was co-transfected into AGS-B cells along with the 1.8-kb hHDC-Luc construct, and the response to H2O2 and PMA was assayed. The DN-EGFR partially blocked H2O2-mediated induction of HDC promoter activity, but had no effect on the PMA response (Fig. 9). In addition, the DN-EGFR construct had no effect on the response to gastrin (not shown).

The GAS-RE Functions as an H2O2-responsive Element—To investigate further the downstream mechanism by which H2O2 activates the HDC promoter, we analyzed a number of HDC deletion constructs for activation by H2O2 treatment. These deletion studies showed that the H2O2-responsive element was located within the basal (–59 to +125) human HDC promoter (data not shown). In previous reports, we mapped the HDC GAS-RE to a 23-bp element located just downstream of the transcriptional start site (26) (Fig. 10A). Ligation of the GAS-RE enhancer upstream of the gastrin-insensitive thymidine kinase promoter in the thymidine kinase-Luc construct
resulted in a 4-fold response to H2O2, slightly less than that seen with PMA (Fig. 10B).

DISCUSSION

Recent studies have suggested that H2O2 can selectively activate a number of genes involved in a variety of biologic responses to oxidant stress, including immunologic, proliferative, and apoptotic responses (15–17, 37, 38). In previous reports, we demonstrated that transcription of the HDC gene was activated by gastrin and PMA through a PKC/ERK/AP-1 pathway (20, 27, 28). We now show that HDC promoter activity can be stimulated by H2O2. The responses were seen at a slightly higher H2O2 concentration (10 mM) than that used in previous studies (3), but may reflect the greater resistance of gastric cells to oxidative stress. The responses were specific for oxidative stress pathways and reactive oxygen intermediates (such as the hydroxyl radical) as shown by the inhibition seen with N-acetylcysteine and mannitol. In addition, the activation of HDC was selective, since no effect of H2O2 was observed on the ODC promoter under similar conditions. Further, we have shown that the downstream target of oxidant stress is the GAS-RE element, which we have previously shown to mediate the HDC transcription response to gastrin and PMA.

Treatment of AGS-B cells with H2O2 led to significant increases in ERK-1 and AP-1 activity. Since earlier studies by our group demonstrated that activation of HDC transcription by gastrin and PMA occurred through ERK- and AP-1-dependent pathways (20, 27, 28), these results strongly suggested that H2O2-induced HDC promoter activation might be mediated through a similar pathway. A role for ERKs in H2O2-dependent HDC promoter activation was confirmed through studies using kinase-deficient ERK constructs. Previous studies by Guyton et. al. (3) reported that the ERKs and AP-1 could be activated by 200 μM H2O2 in both NIH 3T3 cells and PC12 cells through a Ras-dependent pathway, and that ERK activation mediated resistance to H2O2 toxicity. This earlier study also suggested that 200 μM H2O2 treatment moderately stimulated (3–5-fold) JNK activity. In contrast, our results indicated that the JNK pathway was not activated by H2O2 in AGS-B gastric cells, suggesting the possibility of cell-specific differences in the response to oxidant stress. H2O2 treatment stimulated a dramatic increase in the mRNA expression of c-jun and c-fos, similar to that previously observed in NIH 3T3 cells (3). However, in contrast to this earlier study, the induction of c-jun and c-fos gene expression in AGS-B gastric cells was more sustained. Indeed, the greatest levels of c-fos and c-jun gene expression in H2O2-treated AGS-B cells occurred at 120 min, suggesting a more prolonged growth factor response in gastric cells under conditions of oxidant stress.

Gastrin-dependent activation of the HDC promoter in AGS-B cells has previously been shown by our group to occur through a signaling pathway that involves both Raf-1 and MAP kinase (27). However, this gastrin-dependent pathway appears not to utilize Ras but is dependent on activation of PKCs (20, 27). In contrast, the stimulation of ERK activity by H2O2 in AGS-B cells occurs at least in part through activation of the Ras/EGFR pathway (Fig. 11). The increase in EGFR phosphorylation was observed only with H2O2 stimulation and was not seen with gastrin or PMA stimulation. In addition, expression of the...
Fig. 11. Putative H$_2$O$_2$-dependent signaling pathways leading to HDC promoter activation. H$_2$O$_2$-induced proximal signals activate the EGFR and Ras, resulting in activation of ERK cascades and HDC transactivation. In contrast, gastrin activates the CCK-B/gastrin receptor and PKC; both pathways converge on the level of Raf. (Gα, β, and γ G proteins of the α, β, and γ subfamily; API, activating protein 1).

H. pylori microorganisms, they may also injure the surrounding tissue (1). The role of HDC activation and histamine generation in the response to oxidant stress of the gastrointestinal tract remains unclear, although several studies have suggested that histamine synthesized by HDC may facilitate healing of the gut mucosa (23–25) and inhibit the further generation of ROMs by neutrophils (44, 45). In addition, a role in acid secretion has been suggested by recent studies showing that H$_2$O$_2$ at low concentrations is capable of stimulating acid secretion by sevenfold in isolated gastric mucosa (46). Analysis of HDC promoter activation by H$_2$O$_2$ should provide insight into the molecular events characterizing the adaptive response of gastric cells to oxidant stress.

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REFERENCES

1. Weiss, S. J. (1989) N. Engl. J. Med. 320, 365–376
2. Kehrer, J. P. (1993) Crit. Rev. Taxoced. 23, 21–48
3. Guyton, K. Z., Lii, Y., Gerospe, M., Xu, Q., and Holbrook, N. J. (1996) J. Biol. Chem. 271, 4138–4142
4. Zou, A., Horber, E., Gergely, P., Szics, K., Dombradi, V., and Goldman, R. (1993) Biochem. J. 296, 879–888
5. Whisler, R. L., Goyette, M. A., Grants, I. S., and Newhouse, Y. G. (1995) Arch. Biochem. Biophys. 319, 23–35
6. Hiraiishi, H., Terano, A., Ota, S., Mutoh, H., Sugimoto, T., Harada, T., Razandi, M., and Ivey, K. J. (1994) Gastroenterology 106, 1199–1207
7. Davies, G. R., Simmonds, N. J., Stevens, T. R. J., Sheaff, M. T., Banatvala, N., Laurenson, J. F., Blake, D. R., and Rampton, D. S. (1994) Gut 35, 179–185
8. Baik, S. C., Youn, S. H., Chung, M. H., Lee, W. K., Cho, M. J., Ko, H. G., Park, C. K., Kasai, H., and Rhee, K. H. (1996) Cancer Res. 56, 1279–1282
9. Duque, J. M., Davies, M. J., Mapstone, N. P., Dixon, M. E., Schorah, C. J., White, K. L. M., Chalmers, D. M., and Axon, A. T. R. (1996) Carcinogenesis 17, 559–562
10. Lander, H. M. (1997) FASEB J. 11, 118–24
11. Jacobson, M. D. (1996) Trends Biochem. Sci. 21, 83–86
12. Sen, C., and Packer, L. (1990) FEBS Lett. 259, 732–737
13. Fujimoto, K., Sakata, Y., Tsumada, S., Koyama, T., Morita, H., Ogata, S. I., Matsunaga, C., Getoh, Y., and Iwakiri, R. (1995) J. Biol. Chem. 270, 4138–4142
14. Hocker, M., Koh, T. J., Wang, T. C. (1996) Yale J. Biol. Med. 69, 21–33
15. Bouchier, M., Jung, M. J., and Gerhart, H. (1983) Eur. J. Pharmacol. 96, 129–132
16. Fujimoto, K., Ishimura, I., Granger, D. N., Wada, H., Sakata, T., and Tso, P. (1992) J. Clin. Invest. 89, 126–133
Oxidative Stress Activates Human Histidine Decarboxylase

23054

28. Hocker, M., Zhang, Z., Merchant, J. L., and Wang, T. C. (1997) *Am. J. Physiol.* 272, G522–G530
29. Hill, C. S., Wynne, J., and Treisman, R. (1995) *Cell* 81, 1159–1170
30. Wagner, M., Cao, T., Lopez, M. E., Hope, C., van Nostrand, K., Kobrin, M. S., Fan, H. U., Buchler, M. W., Korr, M. (1996) *Int. J. Cancer* 68, 782–787
31. Robbins, D. J., Zhen, E., Owaki, H., Vanderbill, C. A., Ebert, D., Geppert, T. D., and Cobb, M. H. (1993) *J. Biol. Chem.* 268, 5997–5106
32. Feig, L. A., and Cooper, G. M. (1988) *Mol. Cell. Biol.* 8, 3235–3243
33. Szerebenyi, J., Cai, H., and Cooper, G. M. (1990) *Mol. Cell. Biol.* 10, 5324–5332
34. Cai, H., Erhardt, P., Troppmaier, J., Diaz-Meco, M. T., Sthananandan, G., Rapp, U. R., Moscat, J., and Cooper, G. M. (1993) *Mol. Cell. Biol.* 13, 7645–7651
35. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., Zon, L. I. (1994) *Nature* 372, 794–798
36. Rao, G. N. (1996) *Oncogene* 13, 713–719
37. Pahl, H. L. (1994) *Bioessays* 16, 497–502
38. Bauerle, P. A., Rufer, R. A., Pahl, H. L. (1996) *Pathol. Biol.* 44, 29–35
39. Bae, Y. S., Kang, S. W., Seo, M. S., Baines, I. C., Tekle, E., Chock, P. B., Rhee, S. G. (1997) *J. Biol. Chem.* 272, 217–221
40. Pai, R., Ohta, M., Itani, R. M., Sarfiah, I. J., and Tarnawski, A. S. (1998) *Gastroenterology* 114, 706–713
41. Rao, G. N., Glasgow, W. C., Eling, T. E., and Runge, M. S. (1996) *J. Biol. Chem.* 271, 27760–27764
42. Taniguchi, Y., Taniguchi-Ueda, Y., Mori, K., and Todai, J. (1996) *Nucleic Acids Res.* 24, 3746–3753
43. Dalton, T. P., Li, Q., Bittel, D., Liang, L., and Andrews, G. K. (1996) *J. Biol. Chem.* 271, 26233–26241
44. Ching, T. L., Koelmmah, J. G., and Bae, T. (1985) *Inflamm. Res.* 44, 99–104
45. Miro, A., Chastre, E., Callebert, J., Launay, J. M., Houset, R., Zimer, A., Abita, J. P., and Gespach, C. (1994) *Am. J. Physiol.* 267, R602–R611
46. Bandypadhyay, U., Chatterjee, R., Chakraborty, T. K., Ganguly, C. K., Bhat-tacharya, D. K., and Banerjee, R. K. (1997) *Biochem. Pharmacol.* 54, 241–248