Regulation of CA II and H^+,K^+-ATPase Gene Expression in Canine Gastric Parietal Cells

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The gastric parietal cell is a highly specialized and differentiated cell dedicated to the rapid production of voluminous amounts of HCl in response to the binding of acid secretagogues at specific receptors at the cell surface.[1] Stimulation of this cell by the association of acetylcholine (or an analogue such as carbachol), histamine, or gastrin to its particular receptor initiates a series of intracellular activating events, including the induction of gene expression of proteins involved in morphologic transformation, acid production and secretion, and ionic transport processes.[1,2] Acid secretagogues apparently lead to activation of the gastric proton pump, H^+,K^+-ATPase, resulting in its insertion into the secretory membrane of the cell[2,3] and to the activation of Na^+/H^+ exchange at the basolateral surface[4] as the cell becomes transformed from a nonsecreting to an acid-secreting cell. Associated with the activation of these proton transporters is a large increase in cytosolic pH, which may be handled within the cell via the action of carbonic anhydrase, combining OH^- with CO_2 to form HCO_3^-, followed by the subsequent activation of the basolateral Cl^-/HCO_3^- exchanger.[4,5] Recently, it was shown that both basolateral Na^+/H^+ and Cl^-/HCO_3^- exchange activities are necessary for acid secretion across the apical membrane of these cells.[4]

In a series of studies with freshly isolated canine gastric parietal cells we examined the expression of genes activated by acid secretagogues, using cloned cDNA probes for the proton pump itself, the gastric H^+,K^+-ATPase,[6] and for carbonic anhydrase II (CA II).[7,8] As a probe for a control gene, the expression of which did not vary during the course of our experiments, we used a human cDNA encoding a ubiquitin extension protein, ubiquitin carboxyl-terminal precursor (UBCP).[9,10] Our results indicate that acid secretagogue-specific receptor activation in parietal cells triggers coordinate gene expression of both H^+,K^+-ATPase and CA II and that the induction of CA II gene expression is independent of hydroxyl ion generation resulting from H^+,K^+-ATPase activation.[11]

STEADY-STATE mRNA STUDIES OF H^+,K^+-ATPase AND CA II GENE EXPRESSION

In Northern and dot blotting experiments[10–13] we recently investigated the action of each of the three principal acid secretagogues, carbachol, histamine, and gastrin, on the pattern of expression of genes in gastric parietal cells. Each stimulant rapidly induced the expression of CA II mRNA (FIG. 1).[10] Carbachol (0.1 mM) increased steady-state levels of CA II mRNA to a peak after 20 minutes of incubation, after which there was a decline toward basal mRNA levels within 1

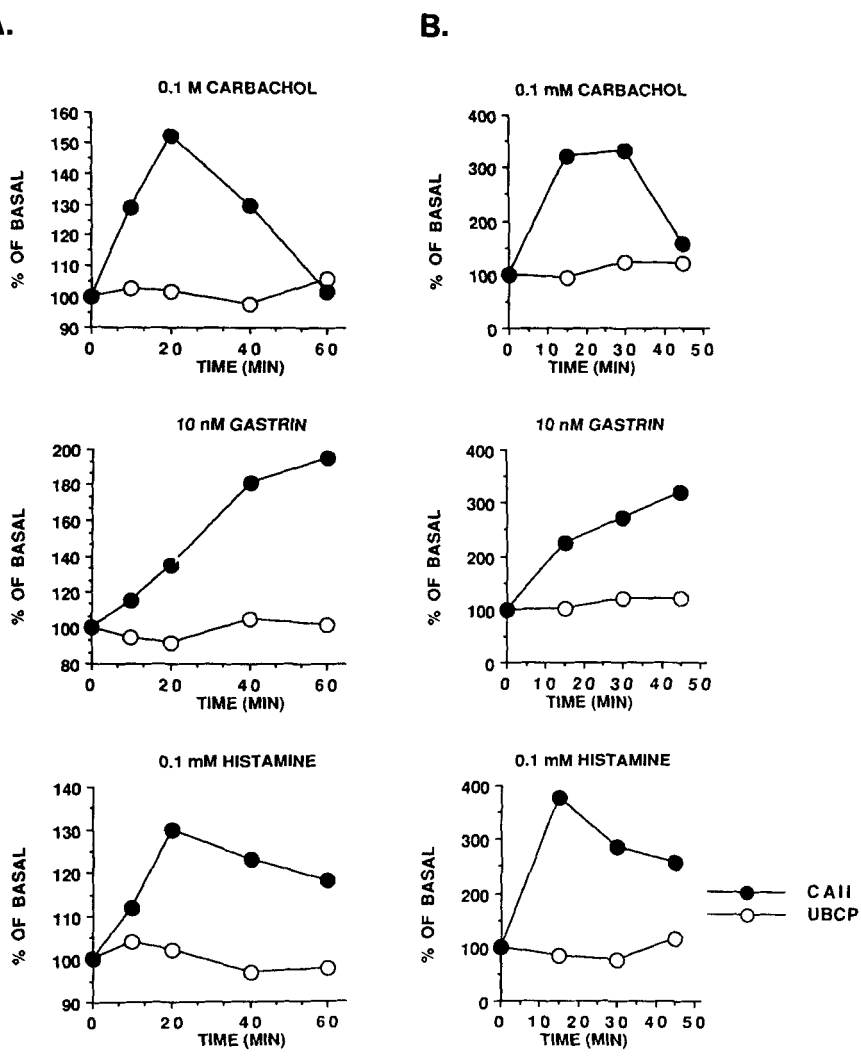

FIGURE 1. Relative changes in **(A)** total mRNA concentrations and **(B)** gene transcription rates of CA II and UBCP with time of incubation in carbachol, gastrin, or histamine. The mRNA data shown, expressed as a percentage of basal, are from cells obtained from a single dog and are similar to results obtained from three other cell preparations. mRNA levels were analyzed by densitometric scanning of dot blots. In other experiments we established the high degree of correlation between hybridizible mRNA quantified by dot blots and by Northern transfers. The relative rate of CA II and UBCP gene transcription for each stimulant, expressed as a percentage of basal, represents the mean value of two separate RNA isolations. Each set of nuclei was assayed in duplicate; thus, each point graphed represents the mean value from four assays. (From ref. 10.)

hour. Similarly, the addition of 0.1 mM histamine to isolated gastric parietal cells induced a modest but reproducible increase over steady-state basal levels in CA II mRNA which peaked around 20 minutes after the addition of secretagogue with a subsequent decline toward basal. In related experiments, when isolated parietal cells were stimulated with 10 nM gastrin (G17), steady-state levels of CA II mRNA exhibited sustained increases that reached levels about twofold over basal within 1 hour. Similar changes in H^+,K^+-ATPase mRNA levels were observed with the various acid secretagogues.

NUCLEAR TRANSCRIPTION STUDIES OF CA II GENE EXPRESSION

To examine whether differences in the observed steady-state levels of CA II mRNA after acid secretagogue addition to parietal cells resulted from differences in transcriptional rates or posttranscriptional events, we performed nuclear runoff experiments.[10,14,15] Data from studies examining the relative transcription levels in nuclei isolated at various times after gastric parietal cells were incubated in each stimulant are shown in FIGURES 1 and 2. CA II gene transcription appeared to reach an early peak between 15 and 30 minutes after the addition of either carbachol or histamine. The increase was transient and decreased rapidly within 45 minutes. Transcription of the CA II gene induced by gastrin increased progressively over the 45-minute period. Moreover, the addition of carbachol, gastrin, or histamine to isolated parietal cells had no effect on either the transcription of UBCP mRNA or its steady-state level.

STUDIES WITH OMEPRAZOLE, A SPECIFIC H^+,K^+-ATPase INHIBITOR

Activation of parietal cell receptors results in the accumulation of H^+ into the secretory canaliculi by an H^+,K^+-ATPase-dependent process. This accumulation is paralleled by a large increase in HCO_3^-, presumably resulting from the action of CA II on the OH^- that accompanies H^+ generation. The HCO_3^- produced is excreted at the basolateral surface of the cell through a Cl^-/HCO_3^- exchanger[5,16] that simultaneously provides the Cl^- needed to accompany H^+ for acid secretion. Having shown that acid secretagogues induce the genes encoding these two enzymes in coordinate fashion, we undertook studies to examine whether the CA II gene is directly induced by secretagogue action or is secondarily induced as a result of the action of the H^+,K^+-ATPase during the process of acid secretion.[12] For these studies we used omeprazole, an agent known to inhibit H^+,K^+-ATPase specifically and irreversibly.[17]

When cells from the same preparation of canine gastric parietal cells were incubated with 0.1 mM omeprazole, levels of both CA II and H^+,K^+-ATPase mRNAs increased nearly twofold over basal. The time course for changes in steady-state levels of CA II mRNA induced by 0.1 mM carbachol showed no difference whether or not the cells had been pretreated with omeprazole. In both instances, CA II mRNA levels reached peaks within 20 minutes of stimulation with carbachol. The increase in H^+,K^+-ATPase mRNA with carbachol stimulation was nearly identical, with or without pretreatment with omeprazole.

Our results led us to conclude that the induction of CA II gene expression with carbachol stimulation is independent of the generation of H^+ (or OH^-) ions by the action of H^+,K^+-ATPase, because the kinetics of induction of CA II mRNA from

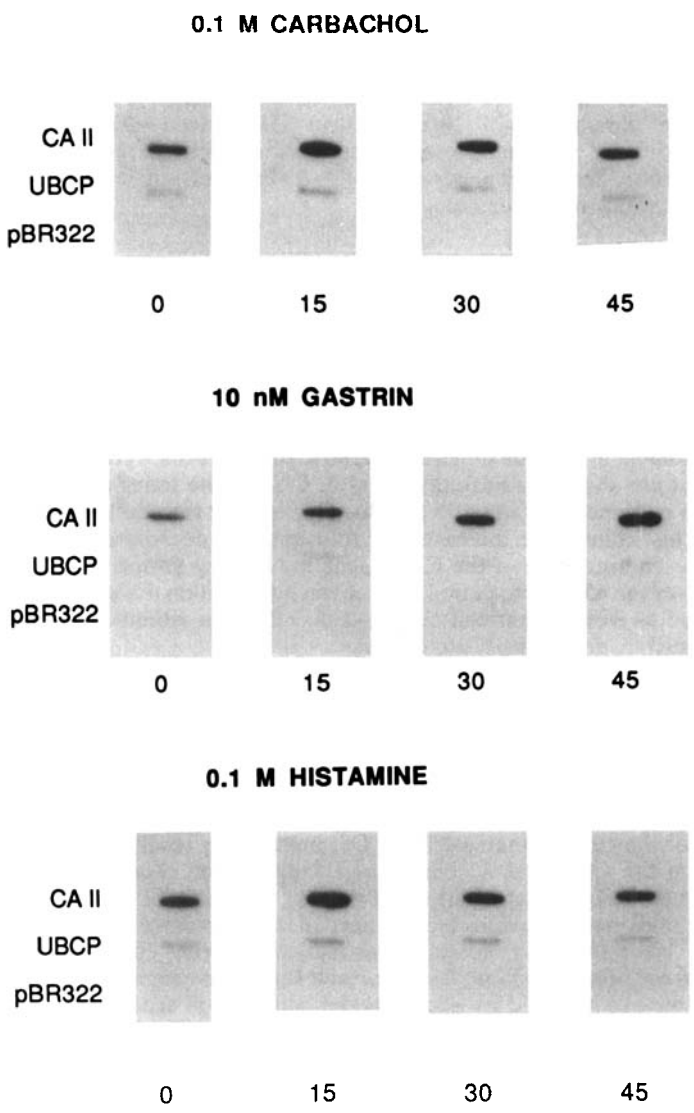

FIGURE 2. Runoff transcription analysis of isolated canine gastric parietal treated with various acid secretagogues. Isolated cells were treated with a single dose of carbachol, gastrin (G17), or histamine for the time indicated. Nuclei were isolated and stored at −70°C until runoff reactions were performed. Approximately equal amounts of purified ^{32}P-labeled RNA transcripts, measured by the number of counts per minute per milliliter of runoff products added to each filter for nuclei that were being directly compared, were hybridized to individual nitrocellulose filter strips containing the indicated DNAs. Quantitation of the hybridization signals was determined by autoradiography followed by densitometry. (From ref. 10.)

the time of carbachol addition are the same whether or not the H^+,K^+-ATPase has been inhibited. Basal levels of H^+,K^+-ATPase and CA II gene expression appear to be dependent on factors different from those in operation in acid secretagogue-stimulated cells. The level of both CA II and H^+,K^+-ATPase mRNA increased almost twofold over basal when parietal cells were incubated for 20 minutes in omeprazole, indicating that the intracellular concentration of the proton pump itself may be important in regulating basal expression of both enzymes.

SUMMARY

Acid secretagogue-specific receptor activation in parietal cells triggers rapid and coordinate gene expression of gastric H^+,K^+-ATPase and of CA II. The rapid rise in steady-state levels of CA II mRNA is due to new transcription of the CA II gene in stimulated cells. Although the presumed function of CA II in activated parietal cells is to catalyze the generation of HCO_3^- from OH^-, regulation of CA II gene expression appears to be independent of the generation of H^+ (and OH^-) through the action of H^+,K^+-ATPase.

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