Acidophylic Cl⁻ and K⁺ Channels of the Gastric Parietal Cell: A New Model of Regulated HCl Secretion

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The gastric parietal cell is responsible for HCl production. Primary active transport of H⁺ occurs through the action of the Mg²⁺-dependent H⁺/K⁺ ATPase, which catalyses the ATP-dependent inward movement of one extracytosolic K⁺ for each H⁺ produced. Pathways for the movement of K⁺ and Cl⁻ are also required for continued action of the H⁺ pump, to provide luminal K⁺ and equivalents of Cl⁻ for HCl production. KCl flux across the apical membrane is under regulation (Sachs et al, 1976; Malinowska et al, 1983). The gastric parietal cell also undergoes a morphological reorganization upon stimulation of HCl secretion, whereupon intracellular vesicles containing the H⁺/K⁺ ATPase (tubulovesicles) disappear, and elaborate into the apical membrane. Since HCl concentrations in the primary gastric secretion exceed 0.15 M, the H⁺/K⁺ ATPase and any other proteins (such as ion channels) required for HCl secretion must function in this harsh environment.

The pathways for K⁺ and Cl⁻ movement are conductive (Cuppoletti & Sachs, 1984; Reenstra & Forte, 1990). In the present work, we summarize recently published data on an acidophylic Cl⁻ channel (Cuppoletti et al, 1993) and new direct evidence for an acidophylic K⁺ channel from electrophysiological (single channel) studies of mammalian (rabbit) gastric apical membranes. Both K⁺ and Cl⁻ channels continue to function when low pH bathes the presumed extracytoplasmic surface of the membrane in which they are embedded. Studies have been carried out on the effects of alteration of membrane potential and asymmetric pH changes on the probability of opening (Po) of the channels. These manipulations mimic the environment of the gastric apical membrane in which the channels must function in concert with the H⁺/K⁺ ATPase for HCl secretion to occur. Our detailed studies of the Cl⁻ channel suggest a model wherein channel activity (as measured by Po) is modulated by changes in the environment of the membranes as the channels are recruited from intracellular stores (tubulovesicles) to the apical membrane. Superimposed on these changes due to altered environment, differences in the Po of the channel of membranes derived from non-secreting (cimetidine-treated) and secreting (histamine-stimulated) rabbit
gastric mucosa were also observed, presumably due to covalent modification such as by cyclic AMP dependent protein kinase. Detailed studies of the K⁺ channel (described here for the first time) are in progress. Comparisons of the properties of the Cl⁻ and K⁺ channels with the characteristics of HCl accumulation suggest that both of these channels may be involved in regulated HCl secretion.

Materials and Methods

Membrane vesicles enriched in the gastric H⁺/K⁺ ATPase from secreting (histamine plus diphenhydramine-injected) or non-secreting (cimetidine-injected) rabbits were prepared as previously described in detail (Cuppoletti & Sachs, 1984). Bilayer reconstitution methods, equipment for channel recording and analysis were carried out as described (Cuppoletti et al, 1993). CsCl and K₂SO₄ solutions were used for Cl⁻ and K⁺ channel current recordings respectively and were buffered to pH 7.4 unless otherwise stated. Measurement of HCl accumulation with acridine orange, ATP hydrolysis, para-nitrophenylphosphatase activity, and protein concentration were carried out as described previously (Cuppoletti & Sachs, 1984).

Results

Evidence for a class of Cl⁻ channels in apical membrane preparations from stimulated rabbit gastric mucosa. Shown in Figure 1 is a single channel current recording obtained after fusion of stimulated rabbit gastric vesicles to planar lipid bilayers. These channels exhibited a linear current-voltage (I/V) relationship, a conductance of 28 pS when recorded in solutions containing 800 mM CsCl, and 7 pS in 150 mM CsCl. The channels were anion selective as judged by the positive reversal potential of +22 mV obtained with a 5 fold gradient of CsCl (800 mM cis, 160 mM trans). The discrimination ratio was 6:1 (Cl⁻:Cs⁺) as calculated using the Goldman-Katz-Hodgkin equation and ionic activities. Anion selectivity of the channel measured under near bi-ionic conditions (with permeability relative to Cl⁻ in parentheses) was I⁻ (2.0) > Cl⁻ (1.0) ≥ Br⁻ (0.9) > NO₃⁻ (0.6) (See Cuppoletti et al, 1993).

Evidence that gastric Cl⁻ channels continue to function at low pH. When the pH of the solution bathing the trans (but not cis) side of the bilayer, was reduced to pH 3.0, single channels persisted (Figure 2). These channels were indistinguishable from those recorded under conditions of symmetrical pH 7.4 solutions with respect to their linear I/V