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Gastric acid secretion in aquaporin-4 knockout mice

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Abstract

The aquaporin-4 (AQP4) water channel has been proposed to play a role in gastric acid secretion. Immunocytochemistry using anti-AQP4 antibodies showed strong AQP4 protein expression at the basolateral membrane of gastric parietal cells in wild-type (+/+) mice. AQP4 involvement in gastric acid secretion was studied using transgenic null (–/–) mice deficient in AQP4 protein. –/– Mice had grossly normal growth and appearance and showed no differences in gastric morphology by light microscopy. Gastric acid secretion was measured in anesthetized mice in which the stomach was lumenally perfused (0.3 ml/min) with 0.9% NaCl containing [¹⁴C]polyethylene glycol ([¹⁴C]PEG) as a volume marker. Collected effluent was assayed for titratable acid content and [¹⁴C]PEG radioactivity. After 45-min baseline perfusion, acid secretion was stimulated by pentagastrin (200 µg · kg^{–1} · h^{–1} iv) for 1 h or histamine (0.23 mg/kg iv) + intraluminal carbachol (20 mg/l). Baseline gastric acid secretion (means ± SE, *n* = 25) was 0.06 ± 0.03 and 0.03 ± 0.02 µeq/15 min in +/+ and –/– mice, respectively. Pentagastrin-stimulated acid secretion was 0.59 ± 0.14 and 0.70 ± 0.15 µeq/15 min in +/+ and –/– mice, respectively. Histamine plus carbachol-stimulated acid secretion was 7.0 ± 1.9 and 8.0 ± 1.8 µeq/15 min in +/+ and –/– mice, respectively. In addition, AQP4 deletion did not affect gastric fluid secretion, gastric pH, or fasting serum gastrin concentrations. These results provide direct evidence against a role of AQP4 in gastric acid secretion.

Keywords

water transport; stomach; gastrin; transgenic mice

Aquaporin-4 (AQP4; mercurial insensitive water channel) is a water-selective transporting protein that was initially cloned from rat lung (9), and, subsequently, homologous cDNAs were isolated from the rat brain and stomach and various mouse and human tissues (11, 19, 34). Immunocytochemistry showed rat AQP4 protein expression at the basolateral membrane of parietal cells in the stomach, as well as in the kidney collecting duct, brain ependyma and astroglia, skeletal muscle sarcolemma, and epithelial cells in the trachea, airways, and colon (6, 7). AQP4 has been shown to be a component of “orthogonal arrays of particles” (OAPs), characteristically square intramembrane particle arrays visualized by freeze-fracture electron microscopy (22, 28, 32). OAPs have been observed at sites of AQP4 expression, including gastric parietal cells (2). Functional analysis showed that AQP4 has substantially higher intrinsic water permeability than other aquaporins (35) and that AQP4

water permeability is not inhibited by mercurial compounds because it lacks a critical cysteine expressed in other aquaporins (24). On the basis of the tissue localization and functional information, it was proposed that AQP4 has a physiological role in gastric acid secretion (5, 23, 30).

Recently, transgenic AQP4-deficient knockout ($-/-$) mice were generated by targeted gene disruption (16). The $-/-$ mice have grossly normal development, growth, and appearance. Functional analysis of kidney collecting duct $-/-$ mice has revealed decreased osmotically driven water permeability (4). Phenotype analysis of the $-/-$ mice has revealed a mild defect in urinary concentrating ability (16), a blunted brain swelling response to water intoxication and ischemic stroke (17), and decreased airspace-capillary water permeability in lung (25). An extensive study of skeletal muscle function showed no abnormalities in AQP4-deficient mice, despite selective AQP4 expression in plasmalemma of fast-twitch skeletal muscle fibers (35).

The purpose of this investigation was to test the hypothesis that AQP4 is required for gastric acid secretion. The involvement of AQP4 in gastric acid physiology was suggested by its specific expression in gastric parietal cells, the cell type responsible for acid production in the stomach and apparent regulation of OAP structure by pentagastrin (2). There are a number of possible mechanisms by which AQP4 could facilitate gastric acid secretion, such as increased glandular fluid secretion, which prevents accumulation of secreted H^+ (5), or an AQP4 transporting role in the parietal cells, such as direct solvent-solute coupling or CO_2 transport. CO_2 transport by the homologous water channel AQP1 has been reported (21); however, recent experiments (33) provided direct evidence against a physiologically important role for AQP1-mediated CO_2 transport. In this study, we compare basal and hormone-stimulated gastric acid production and fluid secretion in wild-type ($+/+$) and AQP4 $-/-$ mice, as well as serum gastrin concentrations and gastric fluid pH.

METHODS

Transgenic mice

Studies were performed in 25- to 35-g $+/+$ and AQP4 $-/-$ mice in a CD1 genetic background. The AQP4 $-/-$ mice were generated by targeted gene disruption as described previously (16). Genotype analysis of tail DNA was performed by PCR when mice were 5 days old. The investigators were blinded to genotype for gastric acid secretion studies, serum gastrin measurements, and immunohistochemistry. Animal protocols were approved by the Stanford University institutional administrative panel on laboratory animal care.

Surgical procedures

Mice were fasted overnight but given free access to 10% dextrose in water. Mice were anesthetized with intraperitoneal pentobarbital sodium (5 mg/kg). An overhead lamp and heating pad were used to maintain core body temperature at 36–38°C. A right jugular vein catheter (PE-10 tubing, Becton Dickinson, Sparks, MD) was inserted via a right neck cutdown, and normal saline was infused at a rate of $10 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Via a midline abdominal incision, a ligature was placed at the esophagogastric junction with care not to injure the vagus nerve trunks. Via a duodenotomy, a flared segment of PE-240 tubing to be used for inflow of perfusate was passed into the stomach and through the wall of the fundus along the greater curve. A PE-240 outflow catheter was passed into the duodenotomy just proximal to the pylorus. Both catheters were secured in place with 6-0 silk suture. Stomachs were flushed with warmed saline until the effluent was clear, followed by perfusions with 0.9% NaCl containing 5 g/l polyethylene glycol (PEG; mol wt 3,000; Sigma, St. Louis, MO) and 5 $\mu\text{Ci/l}$ [^{14}C]PEG (0.3 ml/min). After an initial 15-min stabilization period, effluent

fluid was collected in 15-min intervals. After 45 min, pentagastrin ($200 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was continuously infused intravenously. Effluent was collected for 75 min. In some experiments, intravenous histamine ($0.23 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{ml}^{-1}$) plus intraluminal carbachol (added to the luminal perfusate, 20 mg/l) were added to pentagastrin to induce maximal gastric acid stimulation.

Measurement of gastric acid secretion

Titrateable acid content in pregastric infusate and postgastric effluent were measured by manual titration using sodium hydroxide (10^{-3} M) and a pH meter (Cole-Parmer Instrument, Vernon Hills, IL). All samples were run in triplicate, and the average was reported. The difference in titrateable acid content between the infusate and effluent indicated the total acid secreted into the stomach during each 15-min interval.

Measurement of gastric fluid secretion

Aliquots (0.5 ml) of infusate and effluent were assayed for ^{14}C radioactivity in triplicate by liquid scintillation counting (LS 6000SC, Beckman Instruments, Brea, CA). The amount of net water flux (J_w) within the gastric lumen was determined as follows (3): $J_w = V(1 - [^{14}\text{C}]\text{PEG}_{\text{infusate}}/[^{14}\text{C}]\text{PEG}_{\text{effluent}})/W$, where V is the perfusion rate and W is the excised stomach dry weight in grams.

Gastric pH measurements

After overnight feeding, mice were killed at 8 AM, and gastric fluid was immediately sampled for pH determination using strips of pH paper (pHydron, Brooklyn, NY).

Serum gastrin measurements

Mice were fasted overnight with free access to water. Next, mice were anesthetized with intraperitoneal pentobarbital sodium (5 mg/kg) and then exsanguinated via median sternotomy followed by transection of the inferior vena cava. Blood was immediately collected from the chest cavity and centrifuged at 3,000 rpm for 5 min. Serum was collected and frozen at -20°C . Frozen serum samples were sent to the University of California Los Angeles CURE Center for gastrin RIA.

Morphology and immunocytochemistry

Mice were anesthetized, and stomachs were rapidly excised and incubated in 1% formalin for 4 h. Rings of full-thickness stomach were then placed into PBS plus 30% dextrose at 4°C for 12 h. Tissues were then imbedded in OCT compound, and five $1\text{-}\mu\text{m}$ cryostat sections were obtained. Slides were prepared as previously reported (6). Using hematoxylin and eosin-stained slides, we counted the number of parietal cells per gastric pit. Double-labeled immunohistochemistry was performed using goat anti-rabbit FITC conjugated IgG (Life Technologies, Gaithersburg, MD) against rabbit anti-rat AQP4 antibodies and goat anti-mouse Texas red-conjugated IgG (Jackson ImmunoResearch Labs, West Grove, PA) against monoclonal $\text{H}^+\text{-K}^+\text{-ATPase}$ antibody (MBL International, Watertown, MA). Dual fluorescence images were obtained using a Molecular Dynamics multiprobe 2010 CLSM confocal microscope interfaced to a Silicon Graphics Indigo2 workstation.

RESULTS

The morphology of gastric pits and expression of AQP4 and $\text{H}^+\text{-K}^+\text{-ATPase}$ were assessed. By light and fluorescence microscopy, there were no gross differences in morphology or in the number of parietal cells within the gastric pits. Figure 1 shows the distribution of AQP4 and $\text{H}^+\text{-K}^+\text{-ATPase}$ along a gastric pit and within a parietal cell of $+/+$ and $-/-$ mice. $\text{H}^+\text{-K}^+$

ATPase is uniformly distributed throughout the length of a gastric pit in mice of both genotypes. In $+/+$ mice, AQP4 is more heavily concentrated at the base with no detectable expression in the neck of the gastric pit. No AQP4 staining was seen in the $-/-$ mice.

To determine if AQP4 is involved in parietal cell acid secretion, several secretory agonists were used to increase gastric acid output in $+/+$ and $-/-$ mice. Figure 2 shows the basal, pentagastrin-stimulated, and pentagastrin, carbachol, and histamine-stimulated gastric acid outputs of $+/+$ and $-/-$ mice. Basal acid secretion was not different between $+/+$ and $-/-$ mice (0.06 ± 0.03 vs. 0.03 ± 0.02 $\mu\text{eq}/15$ min, respectively, $n = 25$). Acid secretion was increased during pentagastrin infusion but not different in $+/+$ vs. $-/-$ mice (0.59 ± 0.14 vs. 0.70 ± 0.15 $\mu\text{eq}/15$ min). Addition of luminal carbachol and intravenous histamine resulted in substantially greater acid secretion, which was not different in $+/+$ vs. $-/-$ mice (7.0 ± 1.9 vs. 8.0 ± 1.8 $\mu\text{eq}/15$ min, $n = 25$). These data suggest that AQP4 is not involved in basal or agonist-stimulated gastric acid output.

Because aquaporins have been implicated in transepithelial water transport in several epithelial tissues (15–17, 20), J_w was compared in $+/+$ and $-/-$ mice. Figure 3 shows basal, pentagastrin-stimulated, and pentagastrin, histamine, and carbachol-stimulated J_w in $+/+$ and $-/-$ mice. Although there was a trend toward greater negative J_w (net secretion) in the $-/-$ mice, there were no significant differences. This absence of a difference in water flux suggests that AQP4 is not important in the total water flux in the stomach under basal and stimulated conditions.

To determine whether AQP4 deletion affected the pH of gastric fluid under normal physiological conditions, gastric fluid was sampled in the morning after overnight feeding and pH was measured immediately using pH paper. In $+/+$ and $-/-$ mice, pH was consistently <1.5 , indicating that AQP4 deletion did not prevent the generation of a very low pH in gastric fluid.

Transgenic mice lacking gastrin/CCK-B receptors have elevated serum gastrin concentrations (14, 18, 31). To determine if AQP4 deletion results in altered serum gastrin levels, serum was collected from fasted $+/+$ and $-/-$ mice. Figure 4 shows that fasting serum gastrin levels for $+/+$ and $-/-$ mice were 43 ± 9 and 30 ± 6 pg/ml, respectively (not significant, $n = 16$). The normal serum gastrin in $-/-$ mice supports the conclusion that AQP4 deletion does not alter in vivo gastric acidification.

DISCUSSION

The goal of this study was to determine whether AQP4 is involved in gastric acid and fluid secretion. This study was motivated by the strong, selective expression of AQP4 in gastric parietal cells, and as mentioned earlier, the diverse set of phenotypic abnormalities documented in AQP4 $-/-$ mice. Immunolocalization studies here in mice confirmed AQP4 protein expression in parietal cells at the base of gastric pits, in agreement with previous data in rats and humans (6, 7, 19). However, AQP4 deletion was not associated with changes in gastric mucosal morphology at the light microscopy level or in the rates of basal or stimulated acid or fluid secretion. Furthermore, AQP4 deletion did not affect the ability of the stomach to generate a highly acidic pH, nor did it affect fasting serum gastrin concentrations. Together these data provide direct evidence against a role for AQP4 in gastric acid production.

Various transgenic mouse strains have shown altered gastric acid secretory capabilities. Mice lacking the gastrin/CCK-B receptor exhibit markedly impaired acid secretion, as well as a tenfold elevated serum gastrin level, indicating a critical role for gastrin in the stimulation of acid secretion (14, 27, 31). Gastrin-deficient mice exhibit no demonstrable

stimulated acid secretory response to histamine, carbachol, or gastrin (8). AQP4 $-/-$ mice show no demonstrable impairment in gastric acid secretion, nor do they exhibit elevated serum gastrin concentrations. Although strong agonist-stimulated gastric acid secretion was found, the rates of secretion in our study were somewhat lower than those reported in some rodent studies and comparable to others. In Urethane- or ketamine plus xylazine-anesthetized $+/+$ mice, basal acid output ranges between 0.1 and 6 $\mu\text{eq}/10\text{ min}$ (8, 10, 18). The lower secretion rates found here may in part be due to the suppressive effect of the intraperitoneal pentobarbital that was administered during the several hours needed to complete each experiment. Pentobarbital is reported to have an inhibitory effect on gastric acid secretion (1, 26). However, because of the substantial agonist-stimulated acid secretion and the high sensitivity of the methods used here, we believe that it is unlikely that subtle defects in gastric acidification in AQP4 $-/-$ mice were missed.

It has been proposed that parietal cells function differently throughout their lifespan (12, 13). Parietal stem cells reside in the neck of the gastric pit. As parietal cells mature, they migrate downward toward the base of the pit. It has been suggested that mouse parietal cells secrete acid in the early phase of their lives on the basis of electron microscopy and [^3H]thymidine radioautographic analyses (12, 13). As they mature toward the base of the pit, parietal cells secrete less acid and more water. Our colocalization studies of AQP4 and $\text{H}^+\text{-K}^+\text{-ATPase}$ are consistent with this theory of dual functionality of the parietal cell during its lifespan. The presence of AQP4 only in the base of the gastric pits suggests a greater role in water transport by these parietal cells compared with the more superficial parietal cells in a pit.

AQP4 has been implicated in water transport in several organ systems including kidney, brain, and lung (4, 16, 17, 25, 29). In the stomach, parietal cells account for only a small fraction of the total epithelial surface area and AQP4 is expressed in only a fraction of parietal cells. Although acid secretion is concurrent with water secretion under stimulated conditions, the relative contribution of fluid secretion from parietal cells is unknown. Our measurement of total gastric J_w was probably not sensitive enough to determine whether a defect in fluid transport exists in the parietal cells of $-/-$ mice. It is possible that such a defect might be detected if fluid transport studies are done on isolated, perfused gastric pits. In any case, it is unlikely that AQP4 plays an important physiological role in total gastric fluid secretion in this regard.

Although our results provide functional evidence against a role for AQP4 in gastric acid production and fluid secretion, they do not address the issue of why AQP4 is strongly expressed at the basolateral membrane in gastric parietal cells. AQP4 may increase basolateral membrane water permeability so as to maintain constant parietal cell volume under conditions where the apical cell surface is exposed to fluids of very different osmolalities. However, the AQP4 $-/-$ mice have apparently normal gastric wall morphology and nutritional status. AQP4 expression in gastric parietal cells may represent a vestigial remnant of an earlier time in which high parietal cell water permeability was required. Our results here underscore the notion that the tissue-specific expression of an aquaporin protein does not ensure physiological significance. A similar conclusion was reached in several recent examples, including AQP4 in skeletal muscle (35), AQP1 and AQP4 in salivary gland (15), and several aquaporins including AQP4 in lacrimal gland (20), where aquaporin deletion was not associated with demonstrable phenotypic abnormalities.

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Fig. 1. Immunolocalization of aquaporin-4 (AQP4) (green) and H⁺-K⁺-ATPase (red) in stomachs of wild-type (+/+) and knockout (-/-) mice (a, apical; b, basolateral). Parietal cells in the head (A), neck (B), and base of gastric pits (C) in +/+ mice and in the base of gastric pits in -/- mice (D).

Fig. 2. Basal, pentagastrin-, and pentagastrin/histamine/carbachol-stimulated gastric acid output in +/+ and -/- mice. Data are means $\pm$ SE; $n = 25$.

Fig. 3. Basal and stimulated net water flux (J_w) in +/+ and -/- mice. Data are means $\pm$ SE; $n = 25$.

Fig. 4.
Fasting serum pentagastrin levels in +/+ and -/- mice. Data are means $\pm$ SE; $n = 16$.