Distribution of aquaporins in the colon of *Octodon degus*, a South American desert rodent

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Gallardo, Pedro, Nancy Olea, and Francisco V. Sepúlveda. Distribution of aquaporins in the colon of *Octodon degus*, a South American desert rodent. Am J Physiol Regul Integr Comp Physiol 283: R779–R788, 2002. First published June 13, 2002; 10.1152/ajpregu.00218.2002.—*Octodon degus* is a desert rodent of northern Chile, adapted to survive with a limited supply of water. This rodent has a high degree of fecal dehydration, related to colon water absorption. With the hypothesis that aquaporins (AQPs) might be present in the colon epithelium of *O. degus* and involved in fluid absorption, we studied colon water absorption in vivo and the distribution of AQPs and Na⁺ transporters by immunocytochemistry. AQP-1 was found in apical and basolateral membranes of surface-absorptive and crypt epithelial cells. AQP-8 was found in the cytoplasm of enterocytes of surface colon. AQP-3 immunolabeling, on the other hand, was absent from the epithelium but present in a subepithelial fibroblast layer, pericryptal cells, and muscularis mucosae. The hydration state did not modify the amount of immunostaining for any of the AQPs. Colon water absorption was markedly decreased by the mercurial agent sae. The hydration state did not modify the amount of im-

water absorption is thought to be driven by an osmotic gradient originating in transepithelial NaCl transport. Apical sodium entry in surface-absorptive cells is mediated by transporters, identified in the mammalian colon at the mRNA and protein level, including the NHE3 isoform of Na⁺/H⁺ exchanger, which operates parallel with the HCO₃⁻/Cl⁻ exchanger. Alternatively, Na⁺ entry is conductive and mediated by ENaC Na⁺ channel. Basolateral Na⁺ exit is mediated through Na⁺-K⁺-ATPase (11). The site of the hyperosmotic compartment is not known.

AQP-1 in crypt cells of rat colon (8), AQP-3 and -4 in basolateral membranes (5), and AQP-2 in apical membranes of surface-absorptive cells (6). Recently, AQP-8 was found in rat colon epithelium. However, immuno-

Aquaporins (AQPs) are thought to be involved in transepithelial water movements in the gastrointestinal tract, although their functional role has not been unequivocally proven (12). Several members of the AQP family have been identified in the mammalian colon epithelium at the mRNA and protein level: AQP-1 in crypt cells of rat colon (8), AQP-3 and -4 in basolateral membranes (5), and AQP-2 in apical mem-

brasotheric processes to surface-absorptive epithelial cells and crypt epithelial cells, respectively (11). This model is presently challenged by results showing NaCl and fluid absorption in isolated crypts from rat distal colon (7).

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The major function of the colon is the absorption or secretion of NaCl and water. Under basal physiological conditions, net NaCl and fluid absorption predominates, whereas secretion occurs under neurohormonal stimulation. The classical model of colonic NaCl transport segregates absorptive and secretory processes to surface-absorptive epithelial cells and crypt epithelial cells, respectively (11). This model is presently challenged by results showing NaCl and fluid absorption in isolated crypts from rat distal colon (7).

The aim of this work is to study water absorption and the distribution of AQPs and sodium transporters
in colon epithelium of a desert rodent with a high capacity of fecal dehydration. It is demonstrated that colonic fluid absorption in vivo is markedly reduced in the presence of the mercurial agent p-chloromercuribenzenesulfonic acid (PCMBs), suggesting a participation of AQPs in this process; fluid absorption rate was not increased in dehydrated degus compared with controls. Both findings may be related to the expression of AQP-1, a constitutive and mercurial-sensitive water channel, in apical and basolateral membranes of surface-absorptive and crypt cells. AQP-8 appears to be cytoplasmatic in surface cells of degu colon. AQP-3 is expressed in subepithelial and pericryptal fibroblasts; its absence from epithelium is in marked contrast with its distribution in the rat.

MATERIALS AND METHODS

Animals and tissues. This study was carried out in male adult degus (180–200 g). All experiments were done according to international regulations for animal care and were approved by the Committee of Animal Bioethics of the Facultad de Medicina, Universidad de Chile. The degus were maintained in individual cages with food and water supplied ad libitum. Food was composed of dried seeds including sunflower, corn, wheat, and oat complemented with dried alfalfa pellets. For water-restriction experiments, degus either received food and water ad libitum or were left without water but with free access to food for a 10-day period. The animals were anesthetized with pentobarbital sodium (60 mg/kg ip); blood and urine samples were taken to measure plasma and urine osmolality. The distal colon, kidneys, and brain were removed and placed in ice-cold PBS (pH 7.4) to be then processed for immunocytochemistry.

Colonic fluid transport. Fluid transport was measured in control and water-deprived animals as described previously (21), using 5% agarose gels prepared in 0.9% NaCl (wt/vol). The animals were anesthetized as described above, the colon was carefully exposed, and its lumen was washed with the same NaCl solution. Preweighed 5-mm-diameter agarose gel cylinders were inserted into the lumen of the descending colon and secured by a ligature at least 1 cm distal to the gel. The colon was returned to the abdominal cavity and left for 1 h before removal. Body temperature was checked and maintained at 37°C throughout. The same experiments were carried out with gels containing 1 mM PCMBs. Fluid absorption was measured from the weight difference after a 1-h period. Fluxes are expressed per square centimeter of gel surface area.

Immunolocalization. Localization of AQPs and sodium transporters was carried out by immunocytochemistry and immunofluorescence. Immunocytochemical studies were carried out in paraplast-embedded tissue sections (6-μm thick), previously fixed in Bouin solution. The steps for immunocytochemistry are described elsewhere (19). The primary antibodies used were as follows: anti-AQP-1, anti-AQP-3, anti-AQP-4, and anti-AQP-8 (diluted to 10 μg/ml; Chemicon), and anti-NHE3 isoform of Na+/H+ exchanger (kindly provided by Dr. M. Knepper, National Institutes of Health). They are all polyclonal antibodies raised in rabbit. Anti-α-1 Na+/K+-ATPase subunit (diluted to 10 μg/ml; Upstate Biotechnology) and anti-smooth muscle α-actin (diluted 1:20; Dako) were monoclonal. Immunoreactive sites were revealed using bio-

Fig. 1. Effect of water restriction on colonic water and Na+ absorption and plasma and urine osmolality in the degu. A: water absorption measured in vivo in animals with free access to water or after 10 days of being thirsted. The absorption was measured from 5% agarose gels made with 150 mM NaCl with or without 1 mM p-chloromercuribenzenesulfonic acid (PCMBs). B: Na+ absorption measured from the same intracolonic gels as in A. C and D: plasma and urine osmolality in control and thirsted degus. Means ± SD, n = 5.
tinylated swine anti-rabbit IgG (Dako), followed by streptavidin-conjugated horseradish peroxidase (HRP); the chromogen used was 3,3’-diaminobenzidine in the presence of hydrogen peroxide (Dako Liquid DAB plus kit, Dako). Alkaline phosphatase-conjugated IgG (Vector) was also used as a secondary antibody. In this case, immunoreactive sites were revealed using the BCIP/NBT substrate kit (Vector). Tissue sections were observed and photographed on a Nikon Optiphot microscope.

For immunofluorescence studies, 6-μm-thick cryostat tissue sections were used. They were fixed in fresh 4% (wt/vol) paraformaldehyde in PBS (pH 7.4) for 30 min and rinsed in the same PBS solution. The blocking step was performed with normal goat serum, diluted 1:20 in PBS. Secondary
antibodies were goat anti-rabbit or anti-mouse cyanine-conjugated IgG (Jackson Immunoresearch), diluted to 12 μg/ml. Laser confocal microscopy was performed on a Carl Zeiss system, and the software instrument was used to merge the images.

Membrane preparation and immunoblotting. Colonic mucosa of degu colon was obtained by gently scraping. Membranes were prepared by differential centrifugation. The tissue was homogenized in 250 mM sucrose, 10 mM triethanolamine, 21 μM leupeptin, 57.4 μM PMSF, 0.1 mg/ml aprotinin, pH 7.5. The supernatant of a 3,000-g centrifugation was spun at 100,000 g for 1 h at 4°C. The pellet was resuspended in the same buffer, and protein concentration was determined spectrophotometrically. For immunoblotting, 20 μg protein was solubilized in Laemmli buffer and heated at 65°C for 15 min. Immunoblotting was done in 12% SDS-PAGE minigels. Proteins were blotted into nitrocellulose membranes, blocked for 1 h, washed with TBS-T (pH 7.4), and incubated for 18 h at 4°C with anti-AQP-1 antibody diluted 1:1,000. Membranes were incubated with HRP-conjugated goat anti-rabbit IgG (1:3,000; Jackson Immunoresearch), and immunoreactive sites in membranes were revealed by enhanced chemiluminescence (New England Nuclear).

Ultrastructural studies. Ultrastructural studies were performed through transmission electron microscopy on sections of degu colon. The luminal surface was washed in PBS (pH 7.4) to remove mucous secretion and feces. Samples were fixed in 3% (wt/vol) glutaraldehyde in 100 mM sodium cacodylate buffer (pH 7.4) for 2 h at 4°C and postfixed in 1% (wt/vol) osmium tetroxide in the same buffer during 1 h at room temperature. Tissue samples were embedded in Epon.

Fig. 4. Localization of AQP-3 in distal colon of degu and rat by immunofluorescence and laser-scanning microscopy. A and A’: distribution of AQP-3 immunoreactivity in colonic mucosa of degu and rat, respectively. B and B’: α-1 Na⁺-K⁺-ATPase subunit immunolabeling in distal colon of degu and rat, respectively. C and C’: merged images of A and B and A’ and B’, showing absence of colocalization of AQP-3 Na⁺-K⁺-ATPase in degu and colocalization in rat colon surface epithelium. D and D’: bright field images of the tissue section. Scale bar: 100 μm.
and semithin sections of colon stained with toluidine blue for light microscopy. For transmission electron microscopy, thin sections were double stained with lead citrate and uranyl acetate.

RESULTS

The results of fluid absorption studies are shown in Fig. 1. The effect of water deprivation upon fluid absorption in the distal colon was studied in animals that had been kept without access to water but free access to food for 10 days. Figure 1C shows significantly increased urine osmolality in degus with water restriction without significant change in plasma osmolality, which is consistent with the onset of a renal watersparing mechanism. These changes were not accompanied by changes in the rate of fluid absorption from the colonic lumen measured in vivo. There was a slight increase in water absorption in thirsted degus compared with animals with free access to water (Fig. 1A), but it did not reach statistical significance. Water absorption under both conditions was significantly reduced to similar levels in the presence of PCMBS. The effect of the mercurial agent occurred with no change in the simultaneously measured rate of Na⁺ absorption (Fig. 1B).

AQP-2, a regulated water channel, has been demonstrated in the apical membranes of surface epithelial cells of rat distal colon (6). Immunolocalization experiments in the colon of the degu did not show any AQP-2...
expression. This was not a failure of the antibody used, as a robust AQP-2 label with expected distribution was obtained in parallel degu kidney-immunolabeling experiments (data not shown). The distribution of another AQP, AQP-1, in distal colon of control and dehydrated degus is shown in Fig. 2. Abundant immunostaining was observed in surface and crypt epithelium of control and dehydrated degus. AQP-1 immunolabeling could be observed in apical and basolateral membranes of surface-absorptive cells and also in crypt cells. The intensity and distribution of immunostaining did not appear to differ between the two groups (Fig. 2, A and B). AQP-1 expression in distal colon was also checked by Western blot. Immunoblotting with membranes

Fig. 6. Morphology of degu colonic mucosa. A: light micrograph of a toluidine blue-stained section of colonic mucosa showing surface (se) and crypt epithelium (c) and mm. B: light micrograph of a toluidine blue-stained section of se, white arrows indicate a subepithelial fibroblast layer immediately under surface cells. C: transmission electron micrograph of surface-absorptive cells (×3,000). D: transmission electron micrograph of basal area of se in C showing a fibroblast; the arrow indicates the collagen layer. E: transmission electron micrograph showing the basal area of se; blood capillary (bc), fibroblasts (f), and collagen (c) can be observed. Scale bar in A and B: 100 and 50 μm, respectively; in C: 5 μm; in D and E: 3 μm.
from colon of control and thirsted degus is shown in Fig. 2C. The analysis revealed a band of ~29 kDa with no evidence for an increase in protein abundance upon being thirsted.

Because AQP-8 mRNA has been described in colonic surface epithelium in the rat (4), its presence in the degu was also investigated. AQP-8 was restricted to the surface epithelium with scarce immunostaining over the crypts. There was no apparent difference in the abundance of the staining between control (Fig. 3A) and thirsted (Fig. 3B) animals. In contrast to AQP-1, AQP-8 immunoreactivity was detected in the cytoplasmic compartment of surface enterocytes and seems to be more abundant at the apical pole of the cell. Dehydration did not modify the cellular distribution of AQP-8.

AQP-3 is known to be expressed in the basolateral membranes of rat distal colon epithelial cells (5). AQP-3 localization in degu colon had a completely different distribution compared with that described in the rat. Figure 4A shows that AQP-3 immunostaining was absent from the epithelium but present in a subepithelial cell layer beneath the surface epithelium and encircling the crypts. Simultaneous immunolabeling for Na\(^{+}\)-K\(^{+}\)-ATPase was used as a basolateral marker. As seen in Fig. 4B, this had the expected distribution in basolateral membranes of surface and crypt epithelial cells; no colocalization was found for AQP-3 and Na\(^{+}\)-K\(^{+}\)-ATPase (Fig. 4C). Parallel experiments with kidney sections from degu and rat confirmed the expected (15) localization of AQP-3 in basolateral membranes of distal nephron principal cells (not shown). Figure 4A' shows the distribution of AQP-3 in rat distal colon for comparison. In contrast with the results in degu, AQP-3 was seen to be expressed mainly in basolateral membranes of surface columnar cells. Na\(^{+}\)-K\(^{+}\)-ATPase immunolabeling had the same distribution as in degu colon (Fig. 4B'), being abundantly expressed in basolateral membranes of the whole epithelium. Figure 4C' shows that colocalization of AQP-3 and Na\(^{+}\)-K\(^{+}\)-ATPase was restricted to the basolateral membranes of surface epithelial cells.

Figure 5 shows the distribution of AQP-3 compared with that of smooth muscle \(\alpha\)-actin. In Fig. 5A, the distribution of AQP-3 immunolabeling is shown. In Fig. 5B, labeling of \(\alpha\)-actin identifies the muscularis mucosae (mm), smooth muscle cells surrounding blood vessels (bv), and some lamina propria cells (lp). Superposition of Fig. 5, A and B (see Fig. 5C), revealed that colocalization of AQP-3 and \(\alpha\)-actin occurred mostly in the muscularis mucosae and in the lamina propria. Immunoreactivity for \(\alpha\)-actin was absent from the subepithelial area of high AQP-3 expression (arrow in Fig. 5C).

The structure of degu colonic mucosa was studied to ascertain the nature of the nonepithelial cell type expressing AQP-3 around crypts and under the surface epithelium. Figure 6A shows the structure of the mucosa; the surface epithelium is composed of long columnar cells bearing short microvilli in the apical membrane (Fig. 6C). Crypts of Lieberkühn were seldom seen opening to the lumen and had abundant goblet cells. A subepithelial fibroblast layer can be observed in Fig. 6B as a sheath of cells localized under the surface of the absorptive cells. These fibroblasts probably secrete the collagen fibers that are interposed between the surface cells and the fibroblast (see arrow in Fig. 6D). Figure 6E shows a general view of the area immediately under the basal part of surface cells. The basal part of the surface cells rests on a collagen layer.

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**Fig. 7.** Morphology of degu colon mucosa. A: light micrograph of a toluidine blue-stained section of a crypt encircled by pericryptal fibroblasts (arrow heads). B: transmission electron micrograph of the basal part of a crypt showing pericryptal fibroblasts apposed to epithelial cells (white arrow); the upper right black arrow indicates the crypt lumen. Scale bar in A: 50 \(\mu\)m; in B: 5 \(\mu\)m.
and fibroblasts. A blood capillary is seen under the collagen layer. Figure 7, A and B, shows that fibroblasts are also encircling the crypt perimeter. The electron micrograph in Fig. 7B shows two pericryptal fibroblasts in close apposition, suggesting some kind of barrier function.

AQP-4 could not be detected in degu colon. Parallel control experiments, however, showed AQP-4 immunolabeling in the brain in areas such as the hippocampus and ependymal cells (data not shown), sites of known expression in the rat (9).

Transepithelial fluid transport is driven by osmotic gradients, usually created by transepithelial Na⁺ transport. The importance of apically located Na⁺/H⁺ exchangers has been proposed for the surface and crypt absorptive processes. We therefore investigated the localization of this sodium transporter in the degu colon. Positive immunostaining for the NHE3 isoform of Na⁺/H⁺ exchanger was observed in the apical membrane of surface-absorptive cells (Fig. 8A). As shown in Fig. 8C, Na⁺/H⁺ exchanger and Na⁺/K⁺-ATPase immunolabeling did not colocalize and were distributed at opposite poles of the surface cells. Na⁺/H⁺ exchanger was not detected in the crypts (not shown).

DISCUSSION

The evidence presented here demonstrates that the colon of the degu expresses three members of the AQP water channel family: AQP-1, AQP-3, and AQP-8. AQP-1 immunoreactivity was found in apical and basolateral membranes of both surface-absorptive and crypt cells. There are contradictory reports as to whether AQP-1 is present in rat colon (8, 16). Our results show clear immunostaining of colonic degu sections, and Western blot identifies a protein of the expected molecular weight. The presence of AQP-1 in colonic epithelium strongly suggests that it is involved in transcellular water transport, as it has been demonstrated for the proximal tubule of the mammalian nephron (17). A role for AQP-1 as a water channel in fluid absorption in the colon of the degu is also consistent with the marked reduction of fluid absorption rate in the presence of PCMBS. This mercurial agent is a known blocker of AQP-1 (1). In addition to suggesting the participation of water channels, the inhibition of fluid absorption by PCMBS also suggests that most of the water absorption must occur through a transcellular pathway. Present experiments do not discriminate whether fluid absorption occurs in crypts, surface-absorptive cells, or both sites. The site of fluid absorption has classically been considered to be at the surface epithelium (11). However, experiments with intact epithelium suggest that crypts are involved in fluid ab-
sorption (13, 14). Experiments with isolated microperfused crypts, on the other hand, demonstrate a basal Na+-dependent fluid absorption that can turn into secretion upon appropriate stimulation (7). The surface epithelium of degu expresses the NHE3 isoform of Na+/H+ exchanger at the apical pole of the cells. This exchanger is typically associated with apical membranes of epithelia including the surface-absorptive cells of rat colon (2); its functional coupling with the HCO3/Cl- exchanger allows electroneutral NaCl absorption in the colon (11). Such a NaCl absorption could provide the osmotic gradient for fluid absorption across both membranes of surface cells through AQP-1. In crypt epithelium, AQP-1 may serve for fluid absorption or secretion, depending on the orientation of the osmotic gradient. It must be stressed, however, that our examination of degu colonic morphology provides little evidence for crypt openings that would be required for these glands to participate in transepithelial transport.

AQP-1 immunolabeling was not modified by the hydration state. This is not surprising because constitutive expression of this AQP had been demonstrated in epithelia such as the proximal tubule where it is abundantly expressed in both membrane domains (1). This correlates with an apparent lack of effect of water deprivation upon fluid absorption rate in the colon of the degu. In the rat, dehydration leads to an increase in colonic fluid absorption, which takes place concomitantly with an increased abundance of apical membrane AQP-2 (6). Thus it is possible that the degu has a permanent high level of colonic water absorption and reduced fecal water loss, perhaps related to a more permanent need for water conservation. The rate of water absorption in the colon of the degu was about twice that measured in the rat under identical conditions (6). AQP-2 was absent from the colon of the degu. AQP-1 and -2 antibodies used here recognized the same nephron segments in degu kidney as in the rat (15).

AQP-8 in colonic mucosa was restricted to surface-absorptive cells. In contrast to cellular distribution of AQP-1, AQP-8 was found throughout the cytoplasm, although some apical membrane expression cannot be discarded. This localization is consistent with that described in rat kidney, where AQP-8 immunolabeling was found in cytoplasmatic vesicles of proximal tubule cells (4). It is not known whether these vesicles undergo intracellular trafficking between cytoplasm and the membrane as it happens with AQP-2 (15). We did not observe any apparent change in subcellular distribution of immunoperoxidase labeling between colon of control and dehydrated degus. This suggests that AQP-8 is not regulated in the degu, and its function in transepithelial water transport in colon remains to be established.

A striking difference was found in the distribution of AQP-3 between degu and rat colon. In the degu, AQP-3 had a wide extramembraneous distribution. It was found in a subepithelial cell layer under the surface epithelium, in cells encircling crypts and in smooth muscle cells of the muscularis mucosae. Smooth muscle α-actin and AQP-3 colocalized only in the muscularis mucosae, which suggests that the subepithelial and pericryptal cell layers bearing AQP-3 immunoreactivity are not composed of smooth muscle cells. Ultrastructural studies in degu colonic mucosa showed that the subepithelial and pericryptal cell layers are made up of fibroblasts. These probably secrete the collagen table observed immediately under the basal part of surface epithelium. It has been described that these cells originate from the crypt base and migrate toward the surface epithelium (10). The physiological role of AQP-3 in subepithelial and pericryptal fibroblasts can only be speculated upon. It has been proposed that pericryptal fibroblasts may participate in crypt fluid absorption acting as a relatively low-permeability barrier between the pericryptal space and the lamina propria (13). A pericryptal region of hypertonic Na+ has recently been demonstrated in mouse colon, which could potentially be contained within this cellular sheath (18). According to this model of salt and fluid absorption, the production of a high tonicity pericryptal compartment necessitates a low water permeability of the crypt epithelium and pericryptal sheath. The expression of AQP-1 in both membrane domains of crypts and surface epithelial cells and of AQP-3 in pericryptal fibroblasts would point to a different mechanism of fluid absorption in the colon of the degu. Alternatively, lower hypertonicity might occur in favor of high absorption rates. In rat colon, AQP-3 has been shown to be expressed in basolateral membranes of surface-absorptive cells only (5), and we confirm this finding by demonstrating its colocalization with Na+-K+-ATPase. No evidence for expression in subepithelial or pericryptal structures was found in the rat.

In conclusion, fluid absorption in degu colon is sensitive to mercurial inhibitors, which strongly suggests the participation of AQP water channels. Apical and basolateral AQP-1 may provide a pathway for transcellular epithelial water transport sustained by an osmotic gradient created by sodium transporters. The presence of AQP-8 in surface-absorptive cells needs further investigation to define a role in water absorption. The role of AQP-3 in subepithelial and pericryptal fibroblasts is unknown, and it may represent an adaptation in desert rodents to ensure a high degree of colonic fluid absorption to prevent fecal water loss.

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REFERENCES


