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Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia

(water channel/choroid plexus/ciliary epithelium/bile ducts/intestinal lacteals)

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ABSTRACT **The existence of water-selective channels has been postulated to explain the high water permeability of erythrocytes and certain epithelial cells. The aquaporin CHIP (channel-forming integral membrane protein of 28 kDa), a molecular water channel, is abundant in erythrocytes and water-permeable segments of the nephron. To determine whether CHIP may mediate transmembrane water movement in other water-permeable epithelia, membranes of multiple organs were studied by immunoblotting, immunohistochemistry, and immunoelectron microscopy using affinity-purified anti-CHIP IgG. The apical membrane of the choroid plexus epithelium was densely stained, implying a role for CHIP in the secretion of cerebrospinal fluid. In the eye, CHIP was abundant in apical and basolateral domains of ciliary epithelium, the site of aqueous humor secretion, and also in lens epithelium and corneal endothelium. CHIP was detected in membranes of hepatic bile ducts and water-resorptive epithelium of gall bladder, suggesting a role in bile secretion and concentration. CHIP was not detected in glandular epithelium of mammary, salivary, or lacrimal glands, suggesting the existence of other water-channel isoforms. CHIP was also not detected within the epithelium of the gastrointestinal mucosa. CHIP was abundant in membranes of intestinal lacteals and continuous capillaries in diverse tissues, including cardiac and skeletal muscle, thus providing a molecular explanation for the known water permeability of certain lymphatics and capillary beds. These studies underscore the hypothesis that CHIP plays a major role in transcellular water movement throughout the body.**

Much progress has been made in the quest for carriers of ions and other small molecules, but the molecular mechanism of transmembrane water movement has remained poorly understood (1). Water-selective channels have been proposed to explain the large osmotic water permeability of erythrocytes and certain epithelial cells (2), and the aquaporins, a family of water-transporting proteins, were recently identified in mammals and plants (3).

Discovery of the aquaporin CHIP [channel-forming integral membrane protein of 28 kDa (CHIP28)] (4, 5) and isolation of its cDNA (6) permitted the demonstration of a molecular water channel. This was achieved by expression of CHIP in *Xenopus* oocytes (7) and verified with synthetic proteoliposomes reconstituted with highly purified erythrocyte CHIP (8). Similar to water channels in native membranes (1), CHIP-mediated water transport was reversibly inhibited by $HgCl_2$ reacting with Cys-189 in the CHIP molecule (9). Immunoblot screening revealed abundant CHIP in kidney (4) where it comprises 4% of the total protein in cortical brush-border membrane vesicles (10). Using affinity-purified antibodies specific for the N- and C-terminal domains, detailed immunohistochemistry and immunoelectron microscopy localized CHIP in both apical and basolateral membranes of proximal tubules and descending thin limbs of Henle's loop, a distribution in exact concordance with the known water permeability of the nephron (10). These findings have been confirmed and further defined by others (11–13).

Northern analyses revealed CHIP mRNA in several organs (6, 14, 15). CHIP transcripts were demonstrated in diverse epithelia in fetal rats where three distinct patterns of expression were identified (16). CHIP has been proposed to be the major mechanism by which transmembrane water movement occurs in mammals (3), and this study was undertaken to document the tissue and cellular distributions of CHIP throughout the body.

MATERIALS AND METHODS

Antibodies to CHIP. Immunization of rabbits with highly purified CHIP and affinity purification of antibodies was described (5, 10). Antibodies to human erythrocyte spectrin and band 3 were a gift from Vann Bennett (Duke University Medical Center).

Tissue Preparation for Immunoblots. Four mature Sprague–Dawley rats were injected with 100 units of heparin and killed by ether inhalation, and the carcasses were perfused through the right and left ventricles with 500 ml of 0.15 M NaCl/7.5 mM sodium phosphate/1 mM sodium EDTA, pH 7.5. Selected organs were removed and homogenized in buffer containing 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride, diisopropylfluorophosphate at 0.5 mg/ml, and leupeptin at 4 μg/ml at 0°C as described (4). Membranes were solubilized in SDS, electrophoresed into 12% polyacrylamide gels, and immunoblotted with chemiluminescence autoradiography as described (10).

Immunolocalization of CHIP. Methods were derived from our previous study (10). Albino Wistar rats were anesthetized with pentobarbital (5 mg/100 g) and fixed by vascular perfusion through the left ventricle with 8% paraformaldehyde in 0.15 M sodium cacodylate buffer. Tissue blocks were incubated 2 hr additionally before infiltration for 30 min in 2.3 M sucrose containing 2% paraformaldehyde and then were mounted on holders and rapidly frozen in liquid N_2. Surgical specimens of human tissues immersion-fixed with 3% paraformaldehyde were provided by the Departments of Pathology, Aarhus University Hospital.

Light microscopy was performed on 0.85-μm cryosections or 2-μm paraffin sections after incubation with anti-CHIP (0.07–0.3 μg/ml), visualized with horseradish peroxidase-conjugated secondary antibody, and counterstained with toluidine blue or Mayer's hematoxylin (10). Electron microscopy was performed on 800-Å ultrathin cryosections visualized with protein A-gold (10). Specificity of immunolabeling

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Abbreviation: CHIP, channel-forming integral membrane protein of 28 kDa (CHIP28).

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was confirmed in all tissues with (*i*) nonimmune rabbit IgG, (*ii*) preadsorption of anti-CHIP with a >50-fold molar excess of purified CHIP, and (*iii*) incubation without primary antibody.

RESULTS

Tissue Immunoblots. The presence of CHIP was established in membranes isolated from several rat tissues by immunoblotting with anti-CHIP, an affinity-purified IgG specific for the C-terminal 3-kDa cytoplasmic domain of CHIP (5, 10). Strikingly abundant CHIP was noted in erythrocytes and kidney membranes, while smaller amounts of immunoreactive CHIP were detected in other tissues except whole brain (Fig. 1). The intensity of the immunoblot was stronger with membranes from anterior chamber of eye, lung, and lactating mammary than with membranes from heart, choroid plexus, and liver (Fig. 1). Electrophoretic mobilities of glycosylated CHIP subunits from these tissues were distinct from erythrocyted glycosylated CHIP, and immunoblotting with antibodies specific for spectrin or band 3 revealed negligible erythrocyte contamination.

Distribution of CHIP in Secretory and Resorptive Epithelia. Although anti-CHIP immunostaining was undetectable elsewhere in rat brain, choroid plexus exhibited striking immunostaining restricted to the apical microvilli of epithelial cells in the villous-like processes; basolateral membranes and fenestrated capillaries failed to react (Fig. 2*a*). Specificity of the staining was demonstrated by preadsorption of anti-CHIP with purified CHIP (Fig. 2*a-neg*). Subcellular localization by immunoelectron microscopy with anti-CHIP and protein A-gold revealed immunoreactivity with the inner leaflets of microvillar membranes but not with intracellular or basolateral membranes (Fig. 3*a*).

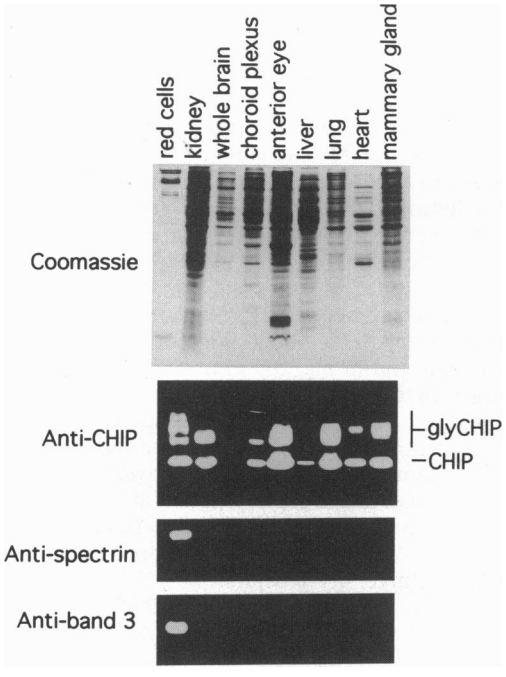

FIG. 1. SDS/polyacrylamide gel electrophoresis and immunoblotting of membranes isolated from various rat tissues. Samples of membrane proteins (10–50 μg) were electrophoresed into 12% polyacrylamide gel slabs and stained with Coomassie blue (*Top*) or immunoblotted with anti-CHIP, anti-erythrocyte spectrin, or anti-erythrocyte band 3. Autoradiographic exposure time of red-cell and kidney lanes was reduced by 90% due to the higher signal intensity. glyCHIP, glycosylated CHIP.

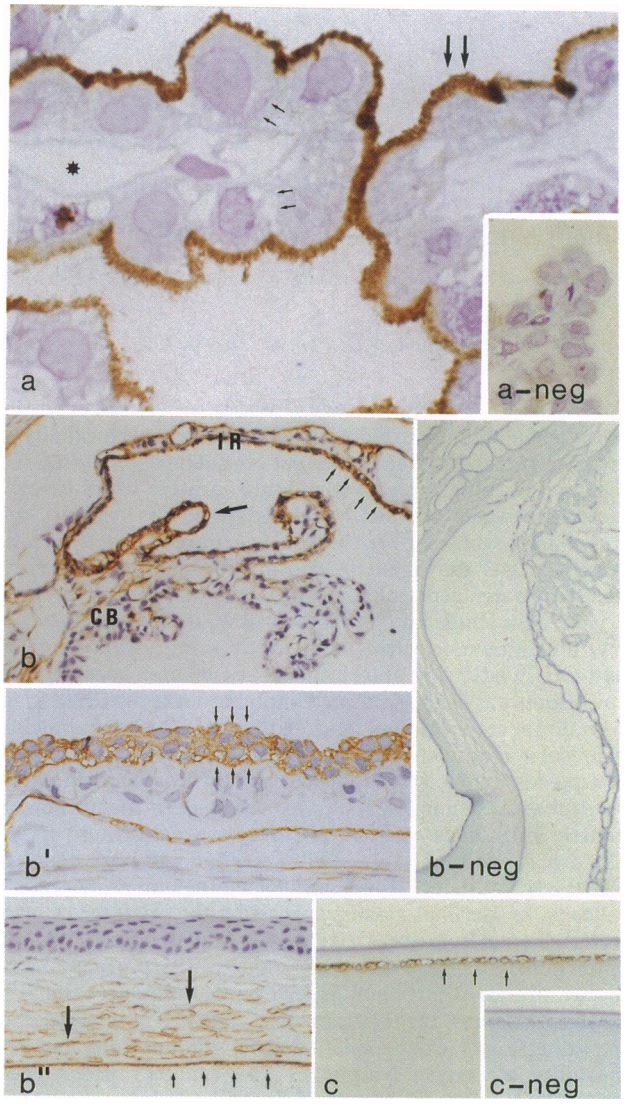

FIG. 2. Immunohistochemical localization of CHIP in choroid plexus and ocular epithelia. Rat tissue sections of 0.85 μm (*a* and *b'*) or 2 μm (other panels) were incubated with anti-CHIP followed with peroxidase goat-anti-rabbit IgG and counterstain. (*a*) Choroid plexus epithelium from the fourth ventricle. Apical microvillar membranes exhibited intense immunoreaction (large arrows), whereas basolateral membranes (arrows) and capillaries (asterisk) were negative. (×775.) (*a-neg*) Preadsorbed control (anti-CHIP was preadsorbed with pure CHIP). (×245.) (*b–c*) Anterior chamber of eye. (*b*) Epithelium covering ciliary body (CB), anterior and posterior epithelium of iris (IR, small arrows), and anterior ciliary process (large arrow) all showed strong immunostaining; posterior ciliary processes were negative. (×45.) (*b'*) Higher magnification showing immunolabeling of apical and basolateral parts of iris epithelium (small arrows). (×200.) (*b-neg*) Preadsorbed control. (×27.) (*b"*) Corneal endothelium (small arrows) was distinctly positive, as were stromal components surrounding tissue clefts (large arrows), but corneal epithelium was negative. (×90.) (*c*) Epithelium over anterior lens (arrows) exhibited immunoreaction with anti-CHIP. (×65.) (*c-neg*) Preadsorbed control. (×36.)

Structures within the anterior chamber of rat eye reacted with anti-CHIP. Iris epithelium and the anterior parts of the ciliary body and processes were immunostained, but the posterior folds were not (Fig. 2*b*). Higher magnification revealed immunoreactive CHIP within the double-layered iris epithelium (Fig. 2*b'*), and immunoelectron microscopy revealed CHIP in the apical and basolateral ciliary membranes (Fig. 3*b*). Corneal endothelium was labeled with

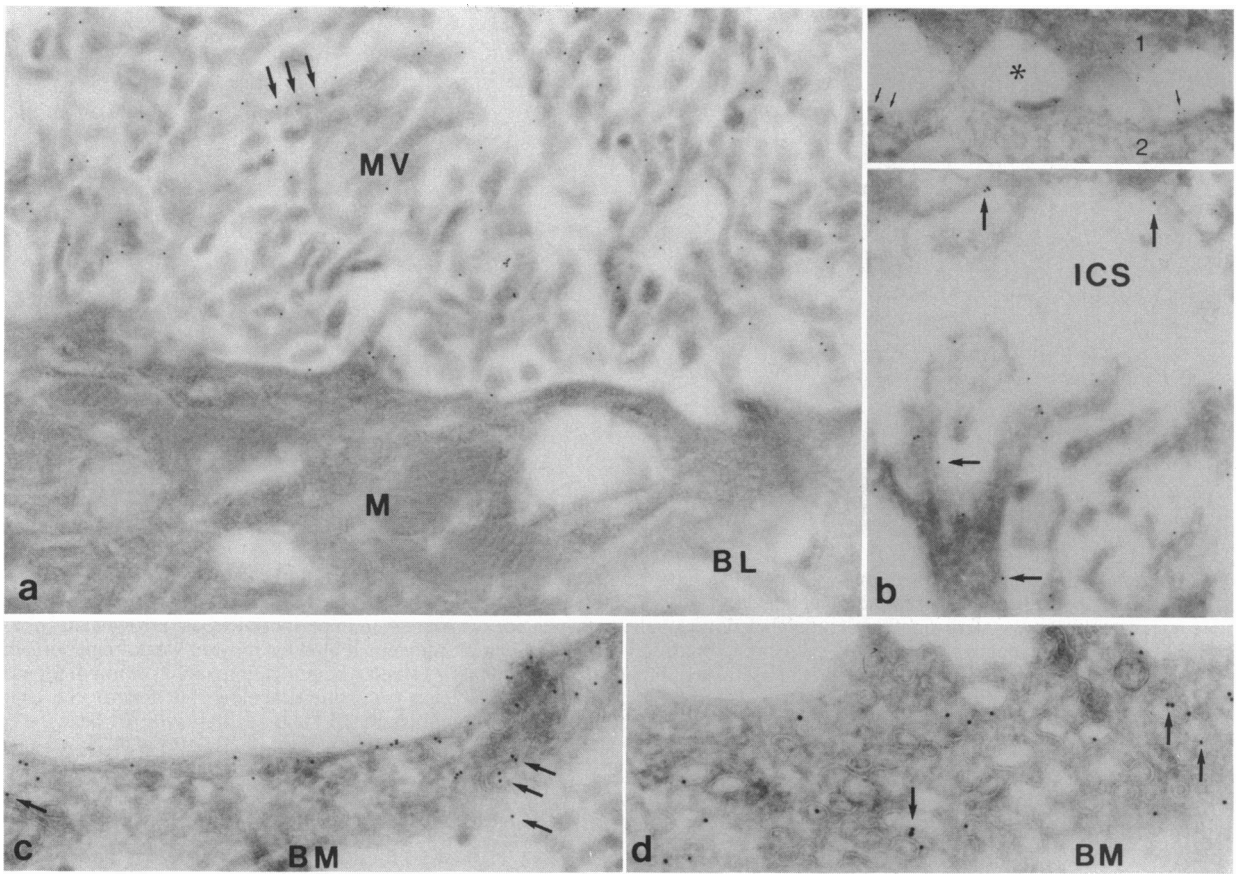

FIG. 3. Subcellular localization of CHIP in rat tissues by immunoelectron microscopy with anti-CHIP and protein A-gold (10 nm). (*a*) Choroid plexus epithelium showed strong immunolabeling of the inner leaflet (arrows) of microvillar membranes (MV), whereas basolateral membranes (BL) and intracellular structures exhibited sparse immunolabeling. M, mitochondrion. (×29,250.) (*b*) Tangential section through basolateral membranes from the inner layer of ciliary epithelium surrounding intercellular spaces (ICS) exhibited strong immunolabeling. (×35,100.) (*Inset*) Apical membranes of two cells (1 and 2) exhibited anti-CHIP immunolabeling. (×21,600.) (*c*) Apical and basolateral membranes (BM) of capillary endothelium from skeletal muscle exhibited immunolabeling (arrows). (×43,200.) (*d*) Plasmalemmal membrane invaginations (caveolae) in tangential section through capillary endothelium were also immunolabeled (arrows); the basement membrane is not well reproduced (BM). (×40,500.)

anti-CHIP, but corneal epithelium was unreactive (Fig. 2*b''*). Also, both apical and basolateral membranes of lens epithelium contained immunoreactive CHIP, whereas lens fiber cells failed to react (Fig. 2*c*). Specificity of these studies was confirmed with preadsorbed anti-CHIP (Fig. 2 *a-*, *b-*, and *c-neg*).

Sections from human liver and neck of human gall bladder were studied, since rats lack gall bladders. Hepatic sinusoids were unreactive, but the continuous endothelium in peribiliary capillary plexus (Fig. 4 *a* and *a'*), interlobular hepatic bile ducts (Fig. 4*a*), and terminal bile ductules (Fig. 4*a'*) were strongly labeled with anti-CHIP. Anti-CHIP immunostaining was weak over the apical membranes but strong over basolateral membranes of gall bladder (Fig. 4 *b* and *b'*). Preadsorbed anti-CHIP failed to react with liver or gall bladder (Fig. 4 *a-* and *b-neg*). Distinct labeling of intestinal lymphatic vessels (lacteals) was observed, whereas epithelium and fenestrated capillaries in small intestine (Fig. 4 *c* and *c'*) and colon (Fig. 4*d*) were not labeled.

Localization of CHIP Within Capillary Endothelia. The strong reaction of immunoblots containing membranes of several tissues is explained by the presence of CHIP reactivity in capillary endothelium rather than with tissue parenchyma. Lung contained abundant anti-CHIP immunoreactivity within capillaries surrounding bronchioles, alveoli, and visceral pleura mesothelium, where the entire circumference of capillaries appeared to be immunolabeled. Although epithelial cells and smooth muscle in bronchiolar airways were clearly not immunostained, reaction with connective tissue and smooth muscle elsewhere in the lung parenchyma cannot be excluded (Fig. 5 *a* and *a'*).

The glandular epithelium of lactating mammary failed to react with anti-CHIP, but capillaries in the surrounding connective tissue exhibited strong immunoreaction (Fig. 5*b*), a distribution shared by pancreas, lacrimal glands, and salivary glands (data not shown). The capillary beds with continuous endothelium within skeletal, cardiac, and smooth muscle were also strongly reactive with anti-CHIP, whereas the myofibrils were unreactive (Fig. 5 *c–e*). Specificity of these capillary immunohistochemical studies was confirmed by the lack of immunostaining with preadsorbed anti-CHIP (Figs. 5 *a-* and *d-neg*). Immunoelectron microscopy confirmed CHIP immunoreactivity over the apical and basolateral membranes of skeletal muscle capillary endothelium as well as small plasmalemmal vesicular structures (Fig. 3 *c* and *d*).

DISCUSSION

This study establishes the locations of CHIP in several secretory and resorptive epithelia and in continuous endothelia of capillaries. The results underscore the hypothesis that CHIP plays a major role in transmembrane water flow throughout the body and provides molecular insight into this phenomenon which occurs in diverse physiologic and pathologic processes.

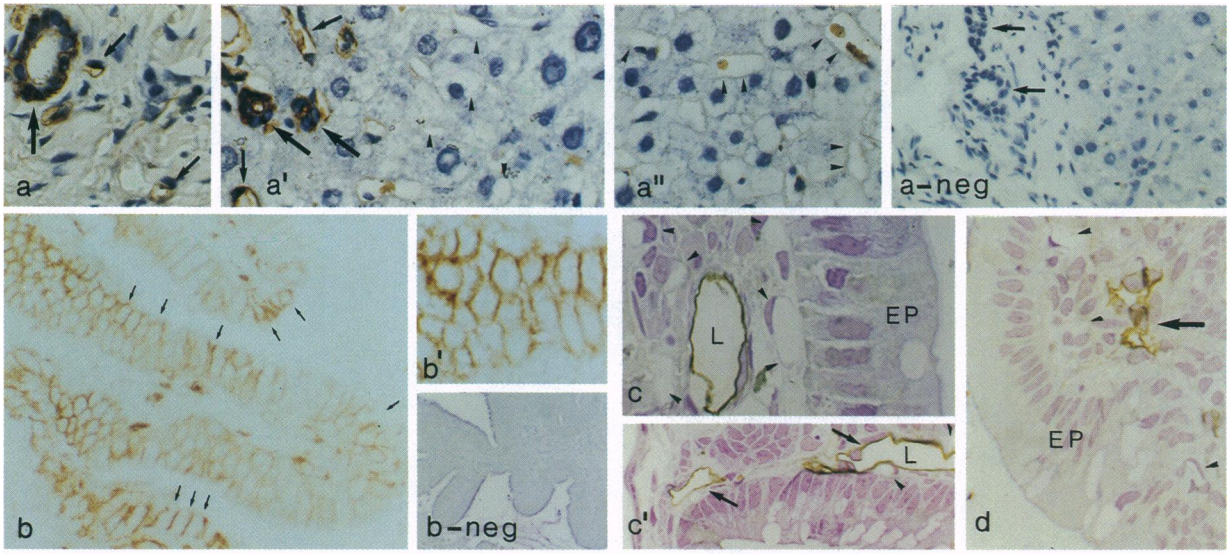

FIG. 4. Immunohistochemical localization of CHIP in 2-μm paraffin sections of human liver and gall bladder and 0.85-μm frozen sections of fixed rat intestine. (*a–a"*) Human liver interlobular bile ducts (large arrows, *a*) and bile ductules (large arrows, *a'*) and nonfenestrated peribiliary capillaries (small arrows) were all distinctly immunolabeled; no labeling of sinusoids was observed in periportal areas (arrowheads, *a'* and *a"*). (×200, ×260, and ×350, respectively.) (*a-neg*) Preadsorbed control. (×90.) (*b*) Neck of human gall bladder showed weak immunoreaction of the apical membrane and strong immunoreaction of the lateral membrane (arrows). (×90.) (*b'*) Higher magnification. (×200.) (*b-neg*) Preadsorbed control. (×23.) (*c* and *c'*) Central lymphatics (lacteals, L) within duodenum (*c*) and ileum (*c'*) exhibit labeling, but fenestrated capillaries (arrowheads) and the epithelium (EP) do not. (×890 and ×300.) (*d*) Rat colon mucosal epithelium (EP) and capillaries (arrowheads) exhibited no immunoreactivity, whereas the central structure (probably a lymphatic, arrow) reacted strongly with anti-CHIP. (×390.)

CHIP was identified at only a single site in brain, the apical microvillar membrane of the choroid plexus epithelium. Na^+,K^+-ATPase is also exclusively located there (17), providing release of Na^+ into the subarachnoid space which drives the passive movement of water into cerebrospinal fluid (18). CHIP was identified at both apical and basolateral

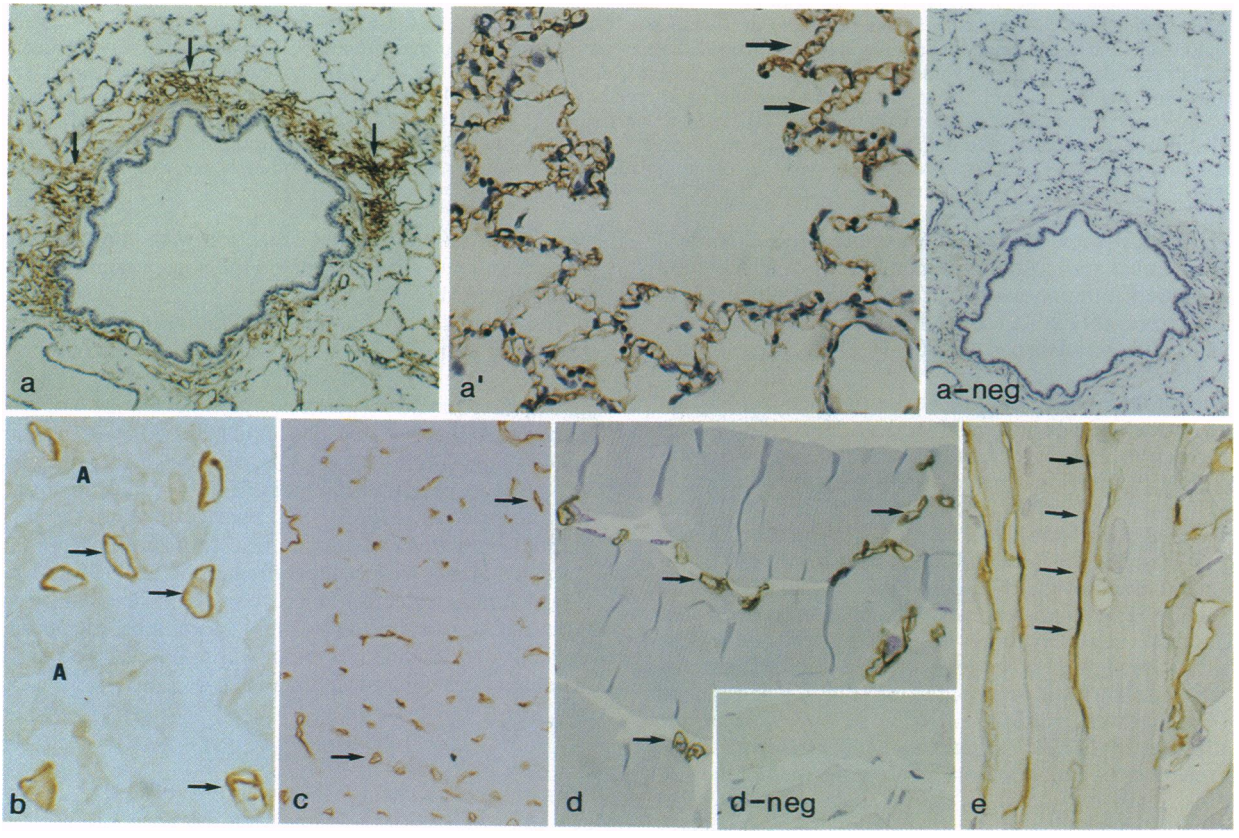

FIG. 5. Immunohistochemical localization of CHIP within capillary endothelia. Strong immunoreactions with anti-CHIP were observed in capillaries (arrows) in 2-μm (*a* and *c*) or 0.85-μm (*b*, *d*, and *e*) sections of rat tissues. (*a*) Cross section of bronchiole and capillary bed. (×80.) (*a'*) Respiratory section of lung. (×180.) (*a-neg*) Preadsorbed control. (×57.) (*b*) Lactating mammary gland with capillaries surrounding alveolar glandular epithelium (A). (×540.) (*c*) Skeletal muscle. (×80.) (*d*) Skeletal muscle at higher magnification. (×270.) (*d-neg*) Preadsorbed control. (×100.) (*e*) Myocardium. (×270.)

membranes of the ciliary epithelium in the eye, where aqueous humor is secreted (19). CHIP is present in the anterior epithelium of lens and in the corneal endothelium, where functional water channels apparently maintain desiccation and transparency of these ocular structures (20). CHIP was localized in both apical and basolateral membranes of intrahepatic bile ductules and ducts, where bile is secreted, and gall bladder epithelium, where bile is concentrated (21). Abundant CHIP exists in apical and basolateral membranes of continuous capillaries of several tissues but not in the parenchyma of muscle or various exocrine glands. These capillaries are known to be water-permeable, balancing the opposing forces of hydrostatic pressure, which drives water into the interstitium, and oncotic pressure, which draws water back into the vascular space (22).

The molecular basis of water transport in each of these phenomena is unsettled, but suggested explanations include transjunctional pores and transmembrane diffusional mechanisms. We propose that CHIP functions in the aforementioned epithelia by conferring high osmotic water permeability responsible for (*i*) secretion of water into cerebrospinal fluid and aqueous humor, (*ii*) removal of water from cornea and lens, (*iii*) secretion of water into bile by intrahepatic biliary ductules and resorption of water from the gall bladder as bile is concentrated, and (*iv*) water release and reuptake by continuous capillaries, which maintain interstitial fluid volume or airway humidity. These studies may also illuminate known pathological processes including the development of effusions or edema due to changes in hydrostatic or oncotic pressures. The abundance of CHIP in the rich plexus surrounding alveolar spaces may explain the rapid water uptake and hemolysis of freshwater drownings.

CHIP is not located at all points where transmembrane water movements occur, and therefore other mechanisms must exist. Fenestrated capillaries probably contain structural pores, and CHIP was not observed in fenestrated capillaries in kidney (10), choroid plexus, lamina propria of intestine, or elsewhere. To avoid hemolysis, gradual water absorption through gastrointestinal mucosal epithelium must occur, and physiologic measurements have demonstrated low water permeability of intestinal epithelium (23), which was shown here to lack CHIP. Intestinal water absorption may therefore occur by diffusional or paracellular mechanisms with rapid dilution into vascular spaces driven by the increased oncotic pressures within the lumens of the intestinal lacteals and capillaries. CHIP was not detected in the renal collecting ducts (10), where the vasopressin-regulated water channel exists (24), or in basolateral membranes of choroid plexus epithelium, in the posterior ciliary processes, or in membranes of several secretory glands, including mammary, salivary, and lacrimal glands. Although this may reflect inaccessibility of antigen, it is more likely that other water channels exist in some of these locations.

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1. Finkelstein, A. (1987) *Water Movement Through Lipid Bilayers, Pores, and Plasma Membranes, Theory and Reality* (Wiley, New York).
2. Solomon, A. K., Chasan, B., Dix, J. A., Lukacovic, M. F., Toon, M. R. & Verkman, A. S. (1983) *Ann. N.Y. Acad. Sci.* **414**, 97–124.
3. Agre, P., Sasaki S. & Chrispeels, M. J. (1993) *Am. J. Physiol.*, in press.
4. Denker, B. M., Smith, B. L., Kuhajda, F. P. & Agre, P. (1988) *J. Biol. Chem.* **263**, 15634–15642.
5. Smith, B. L. & Agre, P. (1991) *J. Biol. Chem.* **266**, 6407–6415.
6. Preston, G. M. & Agre, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11110–11114.
7. Preston, G. M., Carroll, T. P., Guggino, W. B. & Agre, P. (1992) *Science* **256**, 385–387.
8. Zeidel, M. L., Ambudkar, S. V., Smith, B. L. & Agre, P. (1992) *Biochemistry* **31**, 7436–7440.
9. Preston, G. M., Jung, J. S., Guggino, W. B. & Agre, P. (1993) *J. Biol. Chem.* **268**, 17–20.
10. Nielsen, S., Smith, B. L., Christensen, E. I., Knepper, M. A. & Agre, P. (1993) *J. Cell Biol.* **120**, 371–383.
11. van Hoek, A. N. & Verkman, A. S. (1992) *J. Biol. Chem.* **267**, 18267–18269.
12. Zhang, R., Skach, W., Hasegawa, H., van Hoek, A. N. & Verkman, A. S. (1993) *J. Cell Biol.* **120**, 359–369.
13. Sabolic, I., Valenti, G., Verbavatz, J.-M., van Hoek, A. N., Verkman, A. S., Ausiello, D. A. & Brown, D. (1992) *Am. J. Phys.* **263**, C1225–C1233.
14. Lanahan, A., Williams, J. B., Sanders, L. K. & Nathans, D. M. (1992) *Mol. Cell. Biol.* **12**, 3919–3929.
15. Deen, P. M. T., Dempster, J. A., Wieringa, B. & Van Os, C. H. (1992) *Biochem. Biophys. Res. Commun.* **188**, 1267–1273.
16. Bondy, C., Chin, E., Smith, B. L., Preston, G. M. & Agre, P. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4500–4504.
17. Ernst, S. A., Palacios, J. R., II, & Siegel, G. J. (1986) *J. Histochem. Cytochem.* **34**, 189–195.
18. Davson, H., Welch, K. & Segal, M. B. (1987) *The Physiology and Pathophysiology of the Cerebrospinal Fluid* (Churchill Livingstone, Edinburgh), pp. 15–34.
19. Bill, A. (1984) in *Handbook of Physiology*, eds. Renkin, E. M. & Michel, C. C. (Am. Physiological Soc., Bethesda, MD), pp. 1001–1034.
20. Narula, P., Xu, M., Kuang, K. Y., Akiyama, P. & Fischbarg, J. (1992) *Am. J. Physiol.* **262**, C98–C103.
21. Anwer, M. S. (1991) *Prog. Pharmacol. Clin. Pharmacol.* **8**, 3–23.
22. Michell, C. C. (1992) in *The Kidney: Physiology and Pathophysiology*, eds. Seldin, D. W. & Giebisch, G. (Raven, New York), 2nd. Ed., pp. 61–91.
23. van Heeswijk, M. P. E. & van Os, C. H. (1986) *J. Membr. Biol.* **92**, 183–193.
24. Fushimi, K., Uchida, S., Hara, Y., Hirata, Y., Marumo, F. & Sasaki, S. (1993) *Nature (London)* **361**, 549–552.