Localization of Mg$^{2+}$-sensing shark kidney calcium receptor SKCaR in kidney of spiny dogfish, Squalus acanthias

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Hentschel, Hartmut, Jacqueline Nearing, H. William Harris, Marlies Betka, Michelle Baum, Steven C. Hebert, and Marlies Elger. Localization of Mg$^{2+}$-sensing shark kidney calcium receptor SKCaR in kidney of spiny dogfish, Squalus acanthias. Am J Physiol Renal Physiol 285: F430–F439, 2003. First published May 20, 2003; 10.1152/ajprenal.00081.2002.—We recently cloned a homologue of the bovine parathyroid calcium receptor from the kidney of a spiny dogfish (Squalus acanthias) and termed this new protein SKCaR. SKCaR senses alterations in extracellular Mg$^{2+}$ after its expression in human embryonic kidney cells (Nearing J, Betka M, Quinn S, Hentschel H, Elger M, Baum M, Bai M, Chattopyathy N, Brown E, Hebert S, and Harris HW. Proc Natl Acad. Sci USA 99: 9231–9236, 2002). In this report, we used light and electron microscopic immunocytochemical techniques to study the distribution of SKCaR in dogfish kidney. SKCaR antisera bound to the apical membranes of shark kidney epithelial cells in the following tubular segments: proximal tubules (PIa and PIIb), late distal tubule, and collecting tubule/collecting duct as well as diffusely labeled cells of early distal tubule. The highly specific distribution of SKCaR in mesial tissue as well as lateral countercurrent bundles of dogfish kidney is compatible with a role for SKCaR to sense local tubular Mg$^{2+}$ concentrations. This highly specific distribution of SKCaR protein in dogfish kidney could possibly work in concert with the powerful epithelial Mg$^{2+}$-sensing/Mg$^{2+}$-reabsorbing segments. These data provide support for the possible existence of Mg$^{2+}$ cycling in elasmobranch kidney in a manner analogous to that described for mammals.

renal handling of magnesium; transmembrane receptor protein; immunohistochemistry

HOMEOOSTASIS OF DIValent MINERAL ions in body fluids is sustained by the vEB-rate kidney (for a review, see Refs. 14 and 32). Marine elasmobranchs absorb constituent ions of seawater and must excrete them to maintain ionic homeostasis (1, 36). In this regard, Mg$^{2+}$ and Ca$^{2+}$ are excreted principally by the kidney.

In spiny dogfish (Squalus acanthias), urine contains 3 mM Ca$^{2+}$ (a value almost identical to plasma), whereas Mg$^{2+}$ values have been reported to reach 40 mM (plasma ~1 mM) (8). The steep gradient of Mg$^{2+}$ concentration between plasma and urine demonstrates that the kidneys of marine elasmobranchs possess a powerful epithelial Mg$^{2+}$ transport system (5), a major component of which is the second segment of the proximal tubule (PII), where Mg$^{2+}$ secretion is thought to be performed (26, 37).

Previous work by our group has focused on a detailed characterization of physiologically relevant characteristics of the elasmobranch kidney (12, 18, 23, 25). The excretory portion of dogfish kidney consists of multiple, metamERICally formed lobules that grow together during organogenesis (19). Each lobule possesses its own vasculature where renal arteries supply perfusion to glomeruli. The multiple Efferent arterioles merge with the sinusoid capillaries of the renal portal system, which, in turn, are drained via the cardinal veins to the heart. The tubules from each lobule are drained by a single large collecting duct. The lobules are separated into a mesial zone and a zone of lateral bundles. Each nephron is composed of tubular segments that travel in both zones, forming two hairpin loops in the bundles and two extended convolutions in mesial tissue. A schematic drawing of the anatomic organization of a single dogfish kidney nephron is shown in Fig. 1.

Individual nephrons in dogfish kidney possess well-developed proximal and distal segments similar to those described for teleost kidney (21). The proximal tubule is subdivided into two major portions that are designated PI and PII (21). The epithelial cells of PI are endowed with an elaborate apical tubulovesicular apparatus and an extended lysosomal compartment that are similar to those possessed by the entire proximal tubule of mammals (segments S1–S3). By contrast, PII epithelial cells, which apparently have no counterpart in the mammalian kidney, possess an apical compartment filled with clear smooth vesicles that contain high

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concentrations of Mg$^{2+}$ (26). Although present evidence is very limited, data suggest that the PII segment of marine elasmobranchs may engage in net reabsorption of fluid (36), although the PII segment is capable of fluid and salt secretion when proximal tubules from teleosts and elasmobranchs are incubated in vitro (5). In dogfish, the luminal contents of these proximal tubule segments are delivered to the early distal tubule (EDT; the homologue of the thick ascending limb of Henle’s loop) and a late distal tubule (LDT; the homologue of the distal convoluted tubule in mammals) and, finally, to the collecting tubule (CT)/collecting duct (CD).

Because Mg$^{2+}$ uptake in dogfish will vary when they migrate between seawater of different salinities as well as during periods of excessive feeding, Mg$^{2+}$ excretion has to be balanced with alterations in Mg$^{2+}$ uptake to maintain overall Mg$^{2+}$ balance. However, the mechanisms that control either Mg$^{2+}$ secretion in the PII segment or possible reabsorption in the distal tubule segments of elasmobranch kidney are unknown. In this regard, it has been suggested on the basis of histological evidence using $^{26}$Mg$^{2+}$ that renal Mg$^{2+}$ excretion in the euryhaline marine teleost, the killifish (*Fundulus heteroclitus*), is the result of a two-step process where proximal tubule cells secrete Mg$^{2+}$ and Mg$^{2+}$ reabsorption occurs in the CD/CT system (9).

Molecular cloning and characterization of the calcium/polyvalent cation-sensing protein (CaR) in the nephron segments of mammals have opened a new window to an understanding of renal handling of Mg$^{2+}$ (14, 15). Antibody and cDNA probes derived from the sequence of CaRs cloned from mammals have been utilized extensively to identify patterns of cell-specific CaR expression in multiple mammalian tissues (10, 31, 33). These studies have suggested that CaRs possess the ability to “sense” local concentrations of divalent cations and regulate transepithelial ion transport in response to such changes. CaRs are localized to specific cell types in multiple tissues, where they serve as key integrators of divalent mineral ion homeostasis in terrestrial mammals (7, 10, 14, 15, 31, 33). To determine whether CaRs serve similar roles in elasmobranch and teleost fish, we isolated a 4.16-kb shark kidney CaR (SKCaR) cDNA from a dogfish kidney cDNA library (29). SKCaR is a 1,027-amino acid (AA) protein possessing overall 74% AA identity with rat kidney CaR (29). The shark kidney contains two major SKCaR poly A$^+$ transcripts of $\sim$7 and 4 kb that are similar to those in the mammalian kidney (33, 34). A combination of RNA blotting and immunocytochemistry reveals significant SKCaR expression in other shark organs besides the kidney, including rectal gland, stomach, intestine, gill, olfactory lamellae, brain, and ovary (29).

Functional expression of SKCaR protein in human embryonic kidney cells shows that it possesses half-maximal activation (EC$_{50}$) values for Ca$^{2+}$ and Mg$^{2+}$ of $\sim$7.5 and 30 mM, respectively, under mammalian physiological ionic conditions (3, 29). These data suggest that SKCaR likely serves as a Mg$^{2+}$ receptor in the shark kidney. In this study, we hypothesized that the SKCaR protein might possess a highly specific pattern of cellular expression, possibly reflecting its role as an ion sensor in the shark kidney. Using SKCaR-specific antiserum, we report here that SKCaR exhibits a highly specific subsegmental nephron distribution in the shark kidney that is compatible with a role as a principal regulatory sensor for Mg$^{2+}$ homeostasis in elasmobranch kidney.

**MATERIALS AND METHODS**

**Animals.** Male dogfish (*S. acanthias*) were captured by local fishermen in Frenchman Bay for the Mount Desert Island Biological Laboratory during July and August. Ten fish were kept overnight (12–24 h) in large (2,000 liter) tanks with running aerated seawater (average temperature 15°C) before use. Alternatively, four fish were maintained for 1 wk in the tanks. Because the dogfish were wild-caught and did not feed in captivity, it was not possible to determine exactly
when they last fed and the nature of their last meal, the recent site of their occupancy in the ocean, or details of their lives immediately before capture.

**Tissue preparation.** After anesthesia with tricaine (MS 222, Sigma), dogfish were perfused via the heart and truncus arteriosus with heparinized dogfish Ringer solution (in mM: 280 NaCl, 6 KCl, 3 MgCl₂, 5 CaCl₂, 0.5 NaH₂PO₄, 1.0 NaH₂PO₄, 530 urea, 5 glucose, 72 TMAO, and 6 NaHCO₃ in 1 liter). The measured osmolality of this solution was 1,000 mosM. Approximately 400 liters were used to perfuse for 5–10 min at a temperature of 0–4°C and a pressure of 120 cmH₂O. Without a change in pressure and flow, the fixation fluid was added and perfused for 5–10 min.

The fixation fluid contained 2% formaldehyde freshly prepared from paraformaldehyde, 0.05% glutaraldehyde, and 0.5% picric acid in Sorensen’s pH 7.4 phosphate buffer. Sucrose was added to the buffer vehicle (Sorensen’s phosphate buffer, pH 7.4). The osmolality of this solution was adjusted to 850 mosM. After fixation, small tissue pieces were excised from the organ and thoroughly rinsed in Sorenson’s buffer plus 150 mM NaCl, which was adjusted with sucrose to 850 mosM.

To prepare the tissue sections, four methods were used: 1) postfixed with 1% OsO₄ in Sorenson’s buffer, dehydrated via ethanol and acetone, and embedded in Spurr’s medium; 2) embedded in OCT compound, shock-frozen in melting isopentane, and stored in liquid nitrogen; 3) dehydrated via ethanol and xylene and embedded in Paraplast (56°C); and 4) dehydrated via ethanol and embedded in LR-White resin.

**Histology.** Sections (0.5 mm) and thin sections (60 nm) were obtained from tissue blocks (Spurr’s embedding medium) and viewed with a light microscope after being stained with toluidine blue or with an electron microscope (Zeiss EM 902 or Philips EM 301) after being stained with uranyl acetate and lead citrate. The nomenclature of renal structures, i.e., nephron segments, blood vessels, and interstitial cells, was used in accordance with previous results with spiny dogfish and other marine elasmobranch fish (12, 18, 20, 25). In addition to structures, which are generally characteristic of the elasmobranch kidney, spiny dogfish feature a specific subdivision of the second segment of the proximal tubule (PII) (PIIa and PIIb) (12).

**Antibody preparation.** A 17-mer peptide (ARSNSADGRSRPS) was synthesized by standard automated solid-phase techniques, conjugated to keyhole limpet hemocyanin via a cysteine sulfhydryl linkage, and used to immunize rabbits as reported previously (39). Test bleedings were screened by immunoblot analyses to verify antibody binding sites were revealed with the immunoperoxidase technique, involving biotin-streptavidin amplification (ABC Elite Kit, Vector Laboratories, Burlingame, CA) with methyl green as a counterstain. Routine controls were performed 1) with the omission of primary antiserum and 2) with incubation with preimmune serum. Immunoblots containing kidney membranes of epidermal cells and renal tubule cells of distinct nephron subsegments as well as the cytoplasm of selected interstitial cells. By contrast, glomeruli displayed no immunoreactivity. The pattern of antibody binding was confirmed using specific nephron subsegment was present after exposure to corresponding preimmune serum, whereas no chromogenic product was present after exposure to corresponding preimmune serum. A summary of labeling by anti-SKCaR antibody is provided in Table 1.

When immunoblots containing crude membranes isolated from dogfish kidney were probed with affinity-purified anti-SKCaR antibody, prominent bands of 240, 140, and 91 kDa were present (Fig. 2C). These bands were completely ablated after preincubation with an excess of competing peptide. These bands display molecular masses similar to those reported previously for SKCaR protein expressed in human embryonic kidney cells (29) as well as anti-CaR-reactive proteins present in a variety of mammalian tissues (33, 34).

**Immunohistochemistry.** Cryosections (4 μm) and Para-plast sections (2–4 μm) were treated with 0.1% H₂O₂ and used for indirect immunolabeling. After the blocking of unspecific binding with a mixture of 0.2% coldwater fish gelatin, 2% BSA, and 2% fetal calf serum, sections were incubated with primary antiserum (SKCaR antibody; see above), and specific antibody binding sites were revealed with the immunoperoxidase technique, involving biotin-streptavidin amplification (ABC Elite Kit, Vector Laboratories, Burlingame, CA) with methyl green as a counterstain. Routine controls were performed 1) with the omission of primary antiserum and 2) with incubation with preimmune serum. Immunoblots containing kidney membranes of epidermal cells and renal tubule cells of distinct nephron subsegments as well as the cytoplasm of selected interstitial cells. By contrast, glomeruli displayed no immunoreactivity. The pattern of antibody binding was confirmed using specific nephron subsegment was present after exposure to corresponding preimmune serum, whereas no chromogenic product was present after exposure to corresponding preimmune serum. A summary of labeling by anti-SKCaR antibody is provided in Table 1.

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**Immunoelectron microscopy.** Thin sections (60 nm) were obtained from LR-White blocks with an ultramicrotome (Ultrecut E, Leica) and incubated with anti-SKCaR antibody, followed by anti-rabbit IgG-collodial gold conjugate (10-nm gold particles, Aurion-Gent). The sections were counterstained with uranyl acetate and viewed with Zeiss EM 902 and Philips 301 electron microscopes.
The PIa segment in the lateral bundle zone displayed apical SKCaR staining within the region of its brush border (Fig. 3 and see Fig. 6). The PIb segment, which displays multiple bands within mesial tissue near the glomeruli, showed SKCaR-specific staining only at the base of the microvilli of a few cells (Fig. 3).

The PIIa and PIIb segments of the proximal tubule present in mesial tissue displayed markedly different patterns of anti-SKCaR staining characteristics (Figs. 3-5): PIIa cells exhibited no SKCaR staining. In contrast, PIIb cells of all 14 animals studied displayed specific SKCaR immunoreactivity that was observed at their apical membranes (brush border). Interestingly, the intensity of SKCaR labeling of PIIb apical membrane varied greatly among various individual animals studied despite the fact that consistent SKCaR labeling was observed in most other shark nephron segments (see below).

The EDT, which is present exclusively in the lateral bundle zone, is contiguous with the LDT, which thereafter performs numerous bands in mesial tissue. EDT cells were diffusely labeled by anti-SKCaR antiserum (Figs. 3 and 6). The LDT is present in mesial tissue, where it courses along the pathway of PIIa tubules and is frequently in close proximity to both PIIa and PIIb cells (Figs. 3–5). LDT cells in all animals examined showed sharply defined SKCaR

Table 1. Immunoreactivity of SKCaR in the kidney of Squalus acanthias

<table>
<thead>
<tr>
<th>Renal Structure</th>
<th>Localization in Cells</th>
<th>Intensity</th>
</tr>
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<tbody>
<tr>
<td>Glomerulus</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Neck segment</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Proximal tubule</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>PIIa</td>
<td>Apical cell membrane (brush border)</td>
<td>+++</td>
</tr>
<tr>
<td>PIIb</td>
<td>Apical region (below brush border)</td>
<td>+</td>
</tr>
<tr>
<td>Intermediater segment</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Early distal tubule</td>
<td>Diffuse cellular labeling of “intracellular striations”</td>
<td>++</td>
</tr>
<tr>
<td>Late distal tubule</td>
<td>Apical zone (vesicles, cell membrane, glycoalyx)</td>
<td>+++</td>
</tr>
<tr>
<td>Collecting tubule</td>
<td>Apical zone (vesicles, cell membrane, glycoalyx)</td>
<td>– to (+)</td>
</tr>
<tr>
<td>Collecting duct</td>
<td>Apical zone (vesicles, cell membrane, glycoalyx)</td>
<td>+++</td>
</tr>
<tr>
<td>Blood vessels</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Intestinal tissue</td>
<td>Cytoplasm of a subpopulation of cells</td>
<td>+ to ++</td>
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For the nomenclature of the nephron portions in spiny dogfish, see Ref. 12. –, No reaction; (+), faint reaction; + to ++++, weak to very strong reaction.
staining at their apical cell membranes. In addition, only very weak immunostaining was observed at the LDT basolateral membrane in two animals (Fig. 4).

Electron microscopy of LDT cells revealed that they possess short, stubby microvilli with a marked asymmetry of the apical cell membrane, where the extracellular (luminally facing) side was thickened and had a fuzzy coat (glycocalyx). Immunoelectron microscopy of the apical region of LDT cells showed that anti-SKCaR immunoreactivity protein was 1) in the immediate vicinity of the cell membrane; 2) in the apical cytoplasmic region, presumably at apical vesicles; 3) associated with membrane-bound granules located in close proximity to the apical membrane; and 4) outside the cell in the glycocalyx (Fig. 7).

Significant anti-SKCaR immunoreactivity was observed in the CT as well as in the CD (Figs. 3 and 6). The CT at the vascular field of the glomerulus was labeled by the chromogenic reaction. In CT and CD cells, SKCaR antibody binding was confined to the region of the apical cell membrane and its adjacent cytoplasmic zone, where membrane-bound granules abound.

SKCaR staining was also observed in the cytoplasm of small, round cells with large spherical nuclei that were arranged in islets in the interstitium of the lateral bundle zone (Fig. 3). These cells belong to the renal lymphomyeloid tissue that is involved in hematopoiesis of elasmobranch fish (22). Although the function of these cells is presently unknown, they may correspond to hematopoietic cells that possess CaR proteins in mammals (15).

In summary, we consistently found SKCaR labeling in nephron segments Pla, Pb, Piib, EDT, the apical membrane of the LDT, the CT/CD system, and in a subpopulation of cells of the interstitial tissue. However, we observed that SKCaR reactivity was less pronounced in Piib of four animals, and with the exception of two animals, the basolateral membrane of LDT was not labeled.
DISCUSSION

As summarized in Table 1 and schematically in Fig. 1, significant antibody binding against SKCaR was present on the apical cell membranes of the following epithelial cells of dogfish kidney: 1) in the very early portions of PIa at the first hairpin loop in the countercurrent bundles, 2) at the end of PIib in mesial tissue, 3) in the LDT in mesial tissue, 4) in CT at the vascular field of the glomerulus and inside the countercurrent bundles, and 5) in CD. Moderate binding was observed in several cells of PIb. The EDT was also diffusely labeled by anti-SKCaR antibody. It is noteworthy that, in elasmobranchs, cells of the EDT possess extensive amplifications of their basolateral membranes where they form extensive lateral interdigitations running from the cell base to the apical cell junctions (20, 21, 23). Therefore, it is likely that the diffuse labeling pattern observed in dogfish may be due to SKCaR labeling of these extensive basolateral membrane amplifications. However, careful immunoelectron microscopic studies will be necessary to firmly establish this possible SKCaR subcellular localization.

The reason for the variability of anti-SKCaR staining that we observed in the PIib and LDT tubular segments is not presently known. We speculate that this variability could possibly result from wild-caught animals that were in different stages of acclimatization to either captivity or conditions before being captured for study. Further studies are also required to more carefully define any physiological variables that might contribute to differences in SKCaR staining in these tubular segments.

The significance of the observed pattern of SKCaR, a Mg$^{2+}$/H$^{+}$ sensor, for the handling of Mg$^{2+}$ by marine fish

Fig. 4. Cross sections of segments in mesial tissue. Immunostaining of LTD reveals distinct binding sites of antiserum against SKCaR at the apical cell membrane (red). A faint staining can be seen at the basolateral cell membrane forming "intracellular striations.” PIla shows no reaction. SC, sinus capillaries of the renal portal system.

Fig. 5. Section through mesial tissue in the vicinity of glomeruli. Proximal tubule segment PIb with distinct brush border and the 2 portions of the second proximal tubule, PIla and PIib, are shown. These segments are in close contact with the LDT. In this animal, pronounced staining (red) for SKCaR is confined to the LDT and a few portions of the brush border of PIib (arrow).
will be discussed below, with particular reference to the elaborate organization of the elasmobranch kidney. Due to the paucity of transport studies in the elasmobranch nephron (17, 37), these specific SKCaR staining patterns provide the basis for us to propose a functional model of Mg$^{2+}$/H$^{+}$ homeostasis in the elasmobranch kidney that highlights specific aspects of putative recycling of Mg$^{2+}$/H$^{+}$ between Mg$^{2+}$/H$^{+}$-sensing and/or -reabsorbing cells and Mg$^{2+}$/H$^{+}$-secreting cells of the shark's multisegmental nephron. In turn, this model provides a series of testable hypotheses that form the basis of future experiments directed at a more detailed understanding of divalent mineral transport and recycling by the elasmobranch kidney.

**Overall renal handling of Mg$^{2+}$ in marine fish.** Although renal Mg$^{2+}$ excretion is essential for the survival of elasmobranchs in the marine environment because seawater contains a high concentration of Mg$^{2+}$ (~50–60 mM) relative to their plasma Mg$^{2+}$ (plasma ~1–3 mM), functional data quantifying the specific contribution of various nephron segments to this process of net Mg$^{2+}$ excretion are scarce (17, 37). The few measurements of *S. acanthias* kidney show that urinary flow in conscious, restrained spiny dogfish

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**Fig. 6.** Cross section through a countercurrent bundle. The bundle is sectioned near the tip, where the CD leaves and a small CT is entering, coming from a neighbouring bundle (see also Ref. 25). The apical cell membrane of proximal tubule PIa cells (first hairpin loop) is labeled with immunostain (brownish red). Strong binding occurs at the apex of CT and CD cells. EDT of this profile reacts with antibody along the “intracellular striations,” which represent amplifications of the basolateral cell membrane. IS, bundle vein (BV), CV, and bundle artery (BA) appear negative for SKCaR.

**Fig. 7.** Electron micrograph of thin section with immunogold staining of apical region of the LDT. Numerous gold particles (10 nm) are present at the cell membrane, in the fuzzy coat, at a large granule in close proximity to the apical membrane (arrow), and at small apical vesicles, indicating a large amount of SKCaR antigen.
is low (~0.3 ml·h⁻¹·kg⁻¹), <40% of the inulin clearance (~1 ml·h⁻¹·kg⁻¹) (4, 38). In contrast, urinary-to-plasma Mg²⁺ concentration ratios are >40 and indicate net tubular secretion (8).

**Mg²⁺ homeostasis and functional evidence from the proximal and distal tubules.** Secretion of Mg²⁺ occurs in isolated dogfish proximal tubules (35). In previous work, we used quantitative transmission electron microscopy to demonstrate that Mg²⁺ is sequestered at high concentrations within secretory apical vesicles of PI cells in European dogfish (Scyliorhinus caniculus) (26). Micropuncture studies (37) in a related elasmobranch, the little skate (Raja erinacea), show that the final urinary Mg²⁺ concentration is 10-fold higher compared with its plasma value. Transtubular concentration differences (TF/P) for Mg²⁺ are present in many other vertebrates, including teleost fish species, the flounder Pleuronectes americanus and the killifish (Fundulus heteroclitus) (11). Transport kinetics with isolated tubules show saturation far below plasma concentration, suggesting that Mg²⁺ secretion by proximal tubules cannot alone be held responsible for the tight regulation of Mg²⁺ concentration in the plasma of marine fish. Accordingly, Beyenbach and co-workers (5, 6) have suggested that proximal tubules in marine fish can be considered to work as devices for clearance of Mg²⁺ from blood akin to the clearance of plasma solutes by glomerular filtration. Evidence for Mg²⁺ reabsorption in distal tubule segments of marine elasmobranchs is very limited. TF/P values obtained from micropuncture studies of proximal segments vs. CD and final urine of the little skate suggest that more distal nephron segments may be involved in both tubular reabsorption and secretion of Mg²⁺ (37). Although studies of isolated distal tubules of S. acanthias have revealed great similarity to the diluting segment of mammals and other vertebrates (13, 16), no studies of Mg²⁺ transport have been performed to quantify Mg²⁺ transport in this dogfish nephron segment.

**Selected aspects of elasmobranch kidney morphology relevant to Mg²⁺ homeostasis.** The morphological investigations of the kidneys of Chondrichthyans (sharks, skates, and chimeras) have uncovered an extremely complex organization (for references and reviews, see Refs. 12, 18, 20, 25, and 28). Previous comparative anatomic studies in elasmobranchs have revealed the presence of nephron segments that are homologous to corresponding segments in other vertebrates (20). Moreover, it is interesting to note that proximal and EDT segments in kidneys of both mammals and elasmobranchs are spatially separated and their renal tissue is zonated. These patterns of kidney tissue zonation are present in many other vertebrates, including gnathostomes such as Polypteridae and lungfish, and amphibian and higher vertebrates (Sauropsida and Mammalia) (21), and might have originated early in vertebrate evolution.

Thus despite the fact that mammalian and cartilaginous fish kidneys are organized differently, it is intriguing that the kidney of spiny dogfish possesses two renal zones that superficially resemble those of the mammalian renal cortex and medulla, respectively. Mesial tissue of dogfish kidney contains a close association of proximal tubules (segments PIb, PIa, and PIib) as well as EDT. This complement of dogfish nephron segments present in mesial tissue appears to be similar to the mammalian renal cortex with its assembly of proximal tubules, distal convoluted tubule, CT, and cortical CD. By contrast, the lateral bundles of dogfish kidney bear a similarity to the mammalian renal medulla in that the EDT and the CT/CD of dogfish kidney are specially associated like that of the thick ascending limb of Henle's loop and medullary CD of the mammalian kidney. As discussed below, these anatomic associations might be functionally relevant in renal Mg²⁺ handling in the elasmobranch kidney in a manner that is important, as specific nephron segments appear to do so in the mammalian kidney.

**Functional considerations for SKCaR localization in individual elasmobranch nephron segments.** The specific distribution pattern of SKCaR protein in individual cell types present in dogfish nephron segments suggests a unifying hypothesis whereby Mg²⁺ excretion in dogfish is effectively regulated by SKCaR, which senses local Mg²⁺ concentrations. Based on the combination of our previous research on the structural features and Mg²⁺ transport of elasmobranch nephron segments, SKCaR localization data presented here, and published reports of CaRs in mammalian kidneys and cells, we can speculate on the performance of the different segments in dogfish kidney as described below.

The SKCaR protein localized to the apical membrane of dogfish kidney proximal tubule segments PIa and PIb may be involved in the modulation of various cellular transport functions, including transepithelial fluxes of divalent cations as well as perhaps modulating proton secretion. This interesting possibility is suggested by studies of CaR function in rat proximal tubule, where it has been suggested that CaRs might regulate proton secretion and other ion fluxes (33). In this regard, elasmobranch nephrons are well known to be capable of robust proton secretion, resulting in a low pH value of 4 that is maintained throughout the renal tubule. Urinary acidification begins as early as the PIa segment in the countercurrent bundle according to intravital microscopy (27), and low pH is thought to be important in preventing stone formation within the elasmobranch nephron, especially the CT/CD system, where high concentrations of Mg²⁺ salts exist.

PIIa cells likely perform the primary step in Mg²⁺ excretion, yet they do not contain SKCaR protein. PIIa is an exceptionally long segment in mesial tissue of marine Chondrichthyans and provides a significant mass of specialized large epithelial cells. Without the presence of SKCaR, extraction of Mg²⁺ from the sur-

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rounding peritubular circulation may possibly be reg-
ulated by local Mg$^{2+}$ availability. Alternatively, PIla
cell function could be modulated by cross talk with
adjacent SKCaR-containing cells such as PIa or PIlb
cells located in neighboring segments. Future experi-
ments will be necessary to distinguish between these
possibilities or possible regulation of PIla Mg$^{2+}$ secre-
tion by autotcoids.

Present studies of mammalian distal tubule have
emphasized the importance of CaRs to modulate cellu-
lar transport of both divergent (Mg$^{2+}$ and Ca$^{2+}$) as well
as monovalent (Na$^{+}$) cations. Hebert (14) has summa-
rized the salient features of the regulation of thick
ascending limb function by CaRs. Bapty and co-work-
ers (2) further defined renal Mg$^{2+}$ handling by the
study of cultured immortalized mouse distal convol-
uted tubule cells that possess an extracellular polyva-
 lent cation-sensing mechanism responsive to Mg$^{2+}$,
Ca$^{2+}$, and neomycin. In mammals, distal tubules reab-
sorb >15% of filtered Mg$^{2+}$ (32). Thus these data sug-
gest that mammalian distal convoluted tubule exhibits
a renal cell type that both senses and transports Mg$^{2+}$.

It is intriguing to note that the diffuse distribution of
SKCaR in shark EDT cells (perhaps localized to the
basolateral membrane) and apical localization in shark
LDT might correspond to the presence of CaR proteins
on the mammalian medullary thick ascending limb and
distal convoluted tubule (33). By analogy to the
function of the thick ascending limb of Henle’s loop and
distal convoluted tubule in rats, we suggest that EDT
and LDT are very likely segments where Mg$^{2+}$ is
reabsorbed in dogfish. Moreover, Riccardi et al. (33)
have reported the presence of apical punctuate CaR
antibody staining in some type A intercalated cells in
rat CD. Interestingly, dogfish LDT cells display cyto-
plasmic studs and apically located H$^+-$K$^+$-ATPase,
which are characteristically found in mammalian type
A intercalated cells (23).

In a manner similar to the mammalian inner med-
ullary CD (10, 34), the apical membrane of the dogfish
kidney CT/CD system stains intensely with anti-
SKCaR antibody. While the role of SKCaR in dogfish
CT/CD is uncertain, it may modulate NaCl-coupled
water reabsorption, as has been proposed for the floun-
der urinary bladder (29), and prevent excessively high
concentrations of Mg$^{2+}$ within shark urine, as has been proposed for both flounder bladder (29) and mamma-
lian inner medullary CD (10, 34). The overall hypo-
thesis outlined above can be tested by multiple experi-
mental paradigms. A combination of subcellular immu-
nolocalization efforts together with detailed transport
studies of both divergent and monovalent ions including
protons will be facilitated by the labeling pattern of
SKCaR and together provide a better understanding of
divalent cation metabolism in elasmobranch and teleos-
fish as well as Mg$^{2+}$ wasting and stone formation
in mammals.

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