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Expression and Localization of an Aquaporin-1 Homologue in the Avian Kidney and Lower Intestinal Tract

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Abstract

In birds, the kidneys and lower intestine function in osmoregulation. A 271-amino acid homologue to aquaporin-1 (AQP-1) was isolated from the kidneys, cecae, proximal and distal rectum, and coprodeum of the lower intestine in the house sparrow (*Passer domesticus*). This protein has six transmembrane domains connected by two cytoplasmic loops and three extracellular loops. It exhibits 94%, 88%, and 78% homology to AQP-1 sequences of chicken, human and toad, respectively. Many of the highly conserved amino acids that are characteristic of AQP-1 are found in the sparrow sequence. RT–PCR was performed and the presence of AQP-1 mRNA was detected in the kidney and all four regions of the lower intestine. Immunoblots of total protein identified a 28-kDa non-glycosylated AQP-1 band and a 56-kDa glycosylated AQP-1 band in the kidney and all four regions of the lower intestine. Immunohistochemistry demonstrated the presence of the AQP-1 protein within both the renal cortex and medulla. In the lower intestine, the protein was present in the proximal rectum, distal rectum, and in the coprodeum. As AQP-1 functions to allow water movement across mammalian cell membranes, its presence in water-permeable cells in a bird suggests it may have a similar function.

Keywords: Cloaca; Cecae; Protein; Intestine; AQP

1. Introduction

Regulation of water movement across cell membranes is of physiological importance in all organisms. The permeability of some tissues to water is too high to be accounted for by diffusion through the lipid bilayer of cell membranes. In tissues with intrinsically high water permeability, transport proteins account for an enhanced rate of water movement across cell membranes (Finkelstein, 1987). This hypothesis was initially supported by Preston and Agre who discovered the first water channel, *ch*annel-like *i*ntegral *p*rotein, with a molecular weight of 28 kDa (CHIP-28) (Preston and Agre, 1991). Following the recognition of related proteins with similar functions, the Human Genome Nomenclature Committee formally named the emerging family of membrane proteins "aquaporins" (AQP). Aquaporins belong to an ancient and conserved family of membrane integral proteins (MIP) characterized by sequence similarity, the presence of two NPA (Asp-Pro-Ala) motifs, and for their ability to increase the permeability of water in *Xenopus* oocytes (Zardoya and Villalba, 2001). More recently, AQPs have been found to enhance the movement of water across the cell membranes of bacteria, plants and animals (Borgnia et al., 1999).

To date, twelve different AQPs (0–11) have been identified and sequenced in mammals (Hatakeyama et al., 2001; Gorelick et al., 2006). These proteins have been classified into two functional categories: orthodox and multifunctional. Orthodox AQPs are water-selective and include AQP 0, 1, 2, 4, 5, 6 and 10. Multifunctional AQPs also called aquaglyceroporins, transport water, glycerol and some solutes and include AQP 3, 7, 8 and 9 (Verkman and Mitra, 2000). Newly discovered AQP 11 shows low similarity to existing groups and may be a new superfamily (Gorelick et al., 2006). The two main superfamilies of AQPs have been identified in a variety of mammalian tissues and organs including the eye, pancreas, liver, gall bladder, esophagus, salivary glands, kidney and in all regions of the gastrointestinal tract (Borgnia et al., 1999). The distribution of the various AQPs is not uniform in these tissues and is likely to be a reflection of their local function.

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As AQPs facilitate the passive movement of water across biological membranes they would presumably have a physiological role in organs of osmoregulation in non-mammalian vertebrates such as the kidneys and lower intestinal tract, but to date this has not been tested. Non-mammalian vertebrates are able to maintain an adequate level of hydration (Dantzler, 1989). Fish, amphibians and reptiles cannot produce a urine more concentrated than their plasma. Birds are able to concentrate their urine to a modest extent, having a maximum urine to plasma osmolality ratio (U/P) of 2.5, however, under normal conditions birds produce copious amounts of dilute urine (U/P ranges from 0.2 to 1.0) (Dantzler and Braun, 1997). As AQPs are essential in maintaining water balance in mammals, they could also play a role in water balance in non-mammalian vertebrates.

Investigation of non-mammalian tissues that have a naturally occurring high rate of water turnover, such as the kidneys and lower intestine, may shed light on water transport mechanisms. Water and ions flux across the epithelium of avian proximal and distal tubules, and water can flux selectively without ion movement from the collecting ducts (Nishimura et al., 1989). Water is presumed to flux in these tubule segments by diffusion; however, the role of transport proteins in these segments is unknown.

Relatively dilute urine leaves the avian kidney and enters into the urodeum, then within seconds into the coprodeum of the cloaca (Braun and Dantzler, 1972, 1975). As birds do not have a urinary bladder, the cloaca is thought to serve as a temporary storage area for urine. Once in the cloaca, the urine is moved by reverse peristaltic waves of smooth muscle in an orad direction along the length of the rectum as far as the cecae (Brummermann and Braun, 1994). Along its course, water and ions are reabsorbed into the blood. The avian lower intestine has the capacity to absorb a significant volume of fluid generated by the kidney. A study on house sparrows showed that hydrated birds can reabsorb up to 34%, and dehydrated birds up to 52% of the total volume of water entering into the lower intestine (Goldstein and Braun, 1988). Similarly, desert quail can reabsorb up to 50% of the total volume of water entering into the lower intestine (Anderson and Braun, 1985). Thus, in birds, urine modification in the lower intestinal tract represents a significant water conserving mechanism for the animal.

Limited data are available on the expression of aquaporin proteins in birds. Avian AQP-1 has recently been identified and sequenced in the kidney of the Japanese quail and has 82%, 77% and 60% identity to rat AQP-1, frog FA-CHIP, and eel AQP-1 respectively (Yang et al., 2004). A partial sequence (500 bp) for an AQP-1 homologue has also been isolated from hummingbird kidney that has an 83% similarity to bovine AQP-1 (Powers et al., 2002). Our interest lies in the expression and localization of AQP-1 in the avian kidney and lower intestinal tract. Our aim was to identify whether the AQP-1 protein was present in the house sparrow. We used random amplification of cDNA ends (RACE) to identify and isolate an AQP-1 homologue in house sparrows. The nucleotide sequence of this cDNA suggests a protein with high amino acid and structural homology to other vertebrate AQP-1 homologs. RT–PCR, immunoblotting and immunohistochemistry were used to determine the tissue distribution of this AQP-1 homologue in the kidney and lower intestine. Our data demonstrate that AQP-1 is present in the house sparrow kidney and lower intestine.

2. Materials and methods

2.1. Tissue preparation

Seventeen house sparrows, *Passer domesticus*, of mixed gender were collected from the wild and euthanized with an overdose injection of sodium pentobarbital (65 mg/ml). House sparrows were used because we have anatomical data on the kidney and lower intestinal region, suggesting that they act in concert to ensure minimal water loss in this species (Casotti, 2001a,b). The visceral cavity was opened with a mid-ventral incision and the lower intestinal tract was quickly separated from the small intestine just orad to the cecae. For samples processed for immunohistochemistry ($n=8$), the lumen of the incised segment was flushed with and then immersed in half-strength Karnovsky's fixative in 300 mOsm phosphate buffer, pH 7.2. For samples processed for protein extraction ($n=6$), the lumen of the incised segment was flushed with and then immersed in phosphate-buffered saline (PBS) containing 120 mM NaCl, 2.7 mM KCl, 2.7 mM NaH_2PO_4, 25 mM Na_2HPO_4 and protease inhibitor for mammalian tissues obtained from Sigma-Aldrich (St. Louis, MO). For samples used for RNA isolation ($n=3$), the incised segments were flushed with and then immersed in RNAlater™ from Ambion (Austin, TX). Intestinal tissue segments were later subdivided into 4 subsegments consisting of cecae, proximal rectum, distal rectum and coprodeum. The remaining viscera were removed and the dorsal aorta cannulated just cranial to the kidneys. Both kidneys of birds processed for immunohistochemistry were then perfused with half-strength Karnovsky's fixative. As tissue osmolality differs between the renal cortex and the medulla, different osmotic conditions were required for optimal fixation. Eight of the kidneys from 4 birds were perfused with a fixative of low osmolality (300 mOsm) to fix the cortex, and eight kidneys from an additional 4 birds were perfused with higher osmolality fixative (600 mOsm) to fix the medulla. Four kidneys of two birds processed for protein extraction were perfused with ice-cold PBS and those of birds ($n=2$) processed for RNA extraction were perfused with 10 ml of RNAlater™.

2.2. RNA extraction and quantification

Tissue samples used for RNA extraction were stored in RNAlater™ at −20 °C. Total RNA was extracted from 15 to 25 mg of each intestinal segment and kidney tissue using RNAqueous™–4PCR according to the manufacturer's protocol (Ambion). Purified RNA samples were quantified by determining the 260/280 nm absorption ratio using a Beckman DU 530 spectrophotometer (Fullerton, CA). The quality of each RNA sample was assessed by visualization of the 28S and 18S bands on a 1.25% denaturing gel (Sambrook and Russell, 2001).

2.3. Isolation of a cDNA fragment encoding AQP-1

Total RNA (200 ng) from each intestinal segment and the kidney were reverse transcribed into cDNA using an oligo-dT primer (5′GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{24}$-3′) and Superscript III™ (Invitrogen; Carlsbad, CA) in a 20 μl reaction volume at 50 °C for 1 h then at 85 °C for 10 min.

Primers for amplification of a 338 bp AQP-1 fragment from the cDNA were designed by alignment of AQP-1 sequences obtained from *Selasphorus rufus* (Powers et al., 2002) and *Gallus gallus* (Boardman et al., 2002). A sense primer 5′-CTCAGGACAACGTGAAGG-3′ and an antisense primer 5′-GTCTGTGGTGGCAAGGA-3′ were selected from regions where the aligned sequences exhibited 100% homology. The AQP-1 fragment flanked by these primers was amplified using 2 μl of the cDNA reaction product, 1 mM $MgSO_4$, 200 nM dNTP, 200 nM forward primer, 200 nM reverse primer and 2.5 units of Platinum™ *Taq* polymerase (Invitrogen) in a 50 μl reaction volume. A standard β-actin amplicon was used as a loading control (Applied Biosystems). The reaction was heated to 94 °C for 2 min and then subjected to 40 cycles at 94 °C for 30 s, at 55 °C for 30 s and at 60 °C for 30 s. The PCR products were visualized using a 1% agarose gel containing ethidium bromide. The PCR products were also ligated into pCR®4-TOPO® (Invitrogen) and sequenced using both M13 forward and reverse primers (SeqWright, Houston, TX).

2.4. RNA ligase-mediated rapid amplification of cDNA ends (RLM–RACE)

For RLM–RACE, a 5′ gene-specific primer (5′-GGTGGCAAGGACACACAGCACCAG-3′) and a 3′ gene-specific primer (5′-CTTTCAGAGGGAATCAATGCAGGCCAAG-3′) were designed from the sequence of the 338-bp AQP-1 RT–PCR product. Both 5′ and 3′ RLM–RACE were carried out according to the manufacturer's protocol (Invitrogen) using 1 μg of total RNA extracted from the kidney. The product of the 5′ reaction was used in a nested amplification reaction using the antisense primer from the RT–PCR reaction as a gene specific primer. The resulting products of the 5′ nested reaction and the products from the 3′ RLM–RACE reaction were cloned into pCR®4-TOPO® and sequenced using both M13 forward and M13 reverse primers. The two sequences were aligned using BLAST and the resulting full-length cDNA sequence of AQP-1 was determined (Zhang et al., 2000).

2.5. Analysis of the amino acid sequence

The deduced amino acid sequence was determined using MacVector Version 7.2.2 (Accelrys, San Diego, CA). The Kyte–Doolittle hydropathy profile was obtained using TopPred II (Claros and von Heijne, 1994). Amino acid identities between sparrow and other AQP sequences were determined using a BLAST search (Altschul et al., 1997).

2.6. Immunohistochemistry and immunoblot analysis

The AQP-1 primary antibody used for immunoblots and immunohistochemistry was commercially synthesized by Sigma Genosys (The Woodlands, TX). This polyclonal antibody was raised in New Zealand white rabbits against a synthetic peptide corresponding to part of the C-terminal region of AQP-1, CYEEYDLEDDMNSR (252–265). The antigen was then conjugated with keyhole limpet hemocyanin and emulsified with complete Freund's adjuvant. Pre-immune serum was collected on day 0, immunization was carried out on days 1, 14, 28, 42, 56 and 70. Serum was collected on day 77.

The quantity of the peptide-specific antibody present in the serum was determined using ELISA. The reactivity of the peptide-specific antibody with authentic AQP-1 protein was confirmed using kidney tissue homogenates for an immunoblot. Total cellular membrane proteins were extracted from kidney using MBL™ Membrane Extraction (Woburn, MA). The concentration of extracted proteins was determined with a modified Bradford assay using BSA as a standard (Bio-Rad Laboratories, Inc., Hercules, CA) (Bradford, 1976). Total membrane proteins from kidney (4, 8, 12 and 16 μg) along with ProSieve MW standard (Cambrex Bio Science, Rockland, ME) were resolved using 10% SDS–PAGE and then were transferred to Hybond-P membrane (Amersham Pharmacia Biotech, Arlington Heights, IL). The blot was incubated with a 1:50,000 dilution of the serum for 1 h at room temperature in 0.1% PBS containing 1% Tween 20 (PBS-T). The blot was then detected using enhanced chemiluminescence (Amersham Pharmacia Biotech) and a 1:20,000 dilution of an anti-rabbit IgG, peroxidase-linked species-specific whole antibody from donkey in PBS-T. To confirm that the peptide-specific antibody cross-reacted with AQP-1 in tissues, 100 μL of immune serum was incubated with 1 μg of the synthetic peptide for 1 h at room temperature. Following centrifugation at 14,000×*g* for 10 min, the peptide-precipitated serum (PP-serum) was used in place of immune serum on an immunoblot containing kidney homogenate (4, 8, 12 and 16 μg). This blot was then detected as described above. Homogenates of each intestinal area were made using the same procedure as described for the kidney. 20 μg of these homogenates were resolved using SDS–PAGE and the immunoblot procedure as described for the kidney.

For immunohistochemistry, all tissue sections were deparaffinated in xylene, hydrated in a series of graded alcohols, and placed into PBS. Endogenous alkaline phosphatase activity was inhibited by incubation in 0.2 N HCl for 5 min. Nonspecific binding was blocked with 1.5% normal goat serum in PBS containing 0.3% Triton X-100 for 20 min at room temperature. The slides were incubated overnight with a 1:5000 dilution of the peptide-specific serum in a humid chamber at 4 °C. Biotin-conjugated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA) was used at 1:1000 in PBS for 1 h at room temperature. The antibody signal was then amplified with Vector ABC alkaline phosphatase reagent (Vector Laboratories) for 1 h and developed with Vector Red (Vector Laboratories) in PBS for

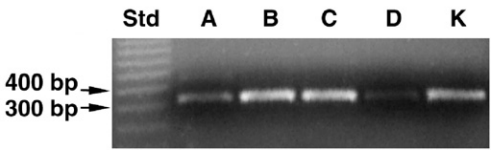

Fig. 1. RT–PCR product from the total RNA of *Passer domesticus*. A 338-bp AQP-1 product was isolated from cecae (A), proximal rectum (B), distal rectum (C), coprodeum (D) and kidney (K). Identity of the PCR amplicon was confirmed by sequencing. A standard β-actin amplicon was used as a loading control (data not shown). Compared to this control, the normalized value of AQP-1/β-actin is 1.22 for the cecae, 1.31 for the proximal rectum, 1.40 for the distal rectum, 1.21 for the coprodeum and 1.48 for the kidney.

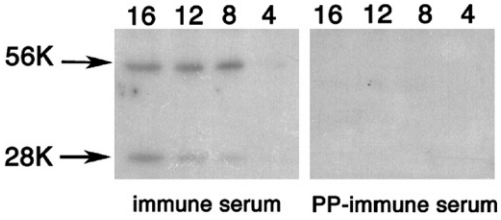

Fig. 3. Immunoblot analysis of AQP-1 from the kidney. The μg amount of total membrane protein loaded in each lane is indicated above each lane. Both serum and peptide-precipitated serum (PP-serum) were diluted 1:50,000 in PBS-T.

30 min at room temperature. Controls included sections incubated with PBS in place of serum, PBS in place of serum and secondary antibody and PBS in place of serum, secondary and avidin/biotin amplification reagents. Additional controls included sections incubated with pre-immune serum in place of serum, and the peptide-precipitated serum in place of serum. After stopping the reactions in distilled water, slides were dehydrated in a series of graded alcohols, cleared in xylene and mounted with Permount. Slides were examined using differential interference contrast (DIC) microscopy on an Olympus BX60 microscope (Melville, NY), and images were captured using a SPOT, Jr. digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI).

3. Results

3.1. Identification of an avian AQP-1 homologue and sequence analysis of the putative AQP-1 protein

Using an alignment of a partial cDNA sequence of *S. rufus* and overlapping cDNA sequence contigs from *G. gallus*, we successfully developed a set of consensus primers that amplified a 338-bp AQP-1 cDNA product from the total RNA of the kidney and each intestinal segment (Fig. 1). From the sequence of this AQP-1 fragment, we then developed a set

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Sparrow MAIEFRKKIFWRAVVAEFLAMSLFIFISIGSALGFNFPVV--ANKTSTRSQDNVKVSLAF 58
Chicken MASEFKKKMFWRAVVAEFLAMILFIFISIGSALGFNFPVS--VNGTSA-TQDNVKVSLAF 57
Human   MASEFKKKLFWRAVVAEFLATTLFVFISIGSALGFKYPVG--NNQTTV--QDNVKVSLAF 56
Toad    MASEFKKKAFWRAVIAEFLAMIFFVFISIGAALGLHFPVQDKANETVSRSQDIVKVSLAF 60
        ** **:** *****:*****  :*:*****:***:::**    * *   :** *******

Sparrow GLSIATMAQSVGHISGAHLNPAVTLGLLLSCQISIFKALMYILAQCLGAVVATAILSGVT 118
Chicken GLSIATMAQSVGHISGAHLNPAVTLGLLLSCQISIFKALMYILAQCLGAVVATAILSGVT 117
Human   GLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISIFRALMYIIAQCVGAIVATAILSGIT 116
Toad    GLAIATMAQSVGHISGAHLNPAVTVGCLLSCQISILKAIMYIIAQCLGAVVATAILSGIT 120
        **:***:*****************:* ********::*:***:***:**:********:*

Sparrow SSLPGNSLGLNALSEGINAGQGLGIEIIATFQLVLCVLATTDRRRNDVSGSAPLAIGLSV 178
Chicken SSLPYNSLGLNALAKGINAGQGLGIEIIATLQLVLCVLATTDRRRNDVSGSAPLAIGLSV 177
Human   SSLTGNSLGRNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDRRRRDLGGSAPLAIGLSV 176
Toad    SNVENNTLGLNGLSTGVTAGQGLGVEIMVTFQLVLCVVAVTDKRRHDISGSIPLAIGLSV 180
        *.:  *:** * *: *:.:*****:**: *:******:*.**:**.*:.** ********

Sparrow ALGHLLAIDYTGCGINPARSFGSALIANNFENHWIFWVGPIIGGASAALIYDFILAPRTS 238
Chicken ALGHLLAIDYTGCGINPARSFGSALIANNFENHWIFWVGPIIGGAGAALIYDFILAPRSS 237
Human   ALGHLLAIDYTGCGINPARSFGSAVITHNFSNHWIFWVGPFIGGALAVLIYDFILAPRSS 236
Toad    ALGHLIAIDYTGCGMNPARSFGMAVVTKNFQYHWIFWVGPMIGGAAAALIYDFILAPRTS 240
        *****:********:******* *::::**. ********:**** *.**********:*

Sparrow DLTDRMKVWTSGQVEEYDLEGDDMNSRVEMKPK 271
Chicken DLTDRVKVWTSGQVEEYDLEGDDMNSRVEM--- 267
Human   DLTDRVKVWTSGQVEEYDLDADDINSRVEMKPK 269
Toad    DLTDRVKVWTSGQLEEYELDGDD-NPRMEMKPK 272
        *****:*******:***:*:.** *.*:**...
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Fig. 2. Deduced amino acid sequence of sparrow AQP-1 in comparison to the sequences of human, toad and chicken AQP-1. Asterisks, colons and periods indicate identical residues, conserved substitutions and semi-conserved substitutions, respectively. Hyphens indicate gaps used for alignment. Underlined sequences indicate transmembrane domains. The highly conserved NPA motifs are shaded. GenBank Accession # AY817455.

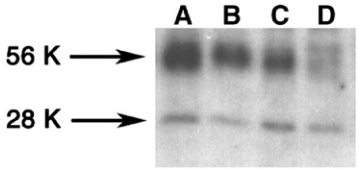

Fig. 4. Immunoblot analysis of AQP-1 in 20 μg of total membrane protein of the cecae (A), proximal rectum (B), distal rectum (C) and coprodeum (D).

of RACE primers that allowed us to isolate overlapping 5′ and 3′ RACE products. The cDNA sequences obtained from these RACE products were merged into a full-length cDNA product of 973 bp that was further confirmed by PCR and sequencing.

Translation of the largest open reading frame of sparrow AQP-1 cDNA yielded a putative sequence spanning 271 amino acids (Fig. 2, GenBank Accession # AY817455). This protein is 94%, 88%, and 78% homologous to the chicken (BBSRC ChickEst database # 603756624F1), human (gi18490903) and toad (gi3821902) sequences respectively. Hydropathy analysis indicates that the sparrow protein, like AQP-1 from other species, contains six putative transmembrane domains connected by two cytoplasmic loops and three extracellular loops. Many of the amino acid residues that are highly conserved in other species are also present in the sparrow sequence (Aoki et al., 2003). The NPA motifs that are characteristic of AQPs are found in the loop connecting the second and third transmembrane domains (residues 72–95) and in the loop connecting the fifth and sixth transmembrane domains (residues 189–212). Also conserved are many of the amino acids involved in AQP-1's structural dynamics including those that establish salt bridges with the negatively charged lipid phosphate groups at Lys_8 and Arg_{12} (Zhu et al., 2001). Those amino acids responsible for the major electrostatic interactions between neighboring monomers are all conserved including the Arg_{12} and the Asp_{230} which stabilize the tetrameric assembly, and the hydrogen bonding between Gly_{30} and Tyr_{188} in the neighboring monomer (Zhu et al., 2001).

3.2. Immunoblot analysis

The expression of AQP-1 protein in the sparrow kidney was investigated using serum raised against the synthetic AQP-1 peptide. On immunoblots of differing concentrations of total membrane protein from the kidney, this serum identified a non-glycosylated AQP-1 band that co-migrated with the 28 kDa standard and the glycosylated forms of AQP-1 that co-migrated with the 56 kDa standard. When PP-serum was used these bands were not evident (Fig. 3).

Immunoblots of intestinal total membrane proteins also identified glycosylated and non-glycosylated forms of AQP-1 in each region of the lower intestine (Fig. 4). The diffuse 56 kDa band seen in Fig. 4 is characteristic of the glycosylated form of AQP-1 that presumably contains branched polylactosaminoglycans. The more focused 28 kDa band found in the kidney (Fig. 4) presumably contains more unbranched polylactosaminoglycans (Agre et al., 1994; Ribatti et al., 2002).

3.3. Immunohistochemistry – kidney

Control sections lacking immune serum showed no immunostaining either in the cortex or the medulla (Fig. 5C). Immunostaining to avian AQP-1 within the cortex was most intense in the distal tubules (Fig. 5A). Some staining was apparent in the glomerular podocytes, and in the brush border of the proximal tubules (Fig. 5A). Immunostaining to AQP-1 was evident in all nephron tubules within the renal medulla and was most intense in the thick limbs of Henle (Fig. 5B). In all cell types within the renal medulla, the cytoplasm and nuclei appeared immunopositive for AQP-1 (Fig. 5B).

3.4. Immunohistochemistry – intestine

All control sections for the cecae, rectum and coprodeum showed no reaction product. These data are not shown but resemble Fig. 5C. AQP-1 was present within the cecae of house sparrows, specifically in the lamina propria (Fig. 6A). The epithelia and the intestinal glands did not immunoreact with the immune serum for AQP-1 (Fig. 6A). AQP-1 was also present in the mucosa of the proximal rectum of house sparrows. Immunostaining was most intense within the lamina propria and submucosa. There appeared to be little if any immunoreaction in the epithelia (Fig. 6B). The muscularis also immunostained for AQP-1 (Fig. 6B). Immunoreactivity for AQP-1 was detected in the epithelium of the distal rectum and in the lamina propria (Fig. 6C). Less intense immunostaining was present in the muscularis (Fig. 6C). Antibodies to AQP-1 also immunostained

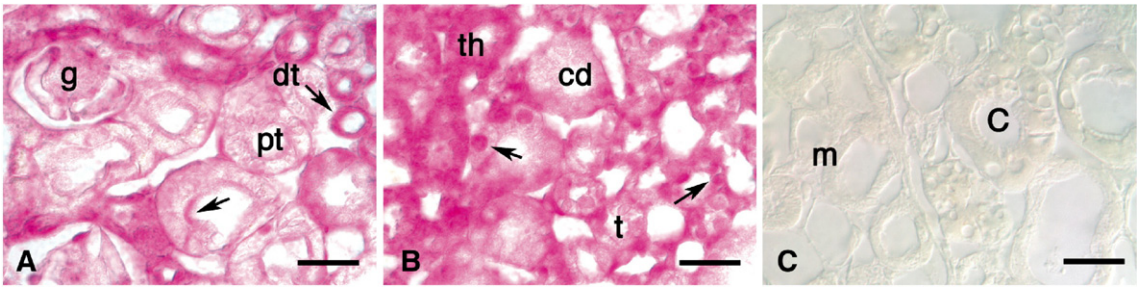

Fig. 5. Light micrographs using differential interference contrast (DIC) showing immunostaining to AQP-1 in the house sparrow kidney. A, cortex; B, medulla, C, negative serum control. g, glomerulus, pt, proximal tubule, dt, distal tubule, th, thick limb of Henle, t, thin limb of Henle, cd, collecting duct, c, cortex, m, medulla, arrowed, nuclei immunostained for AQP-1. Scale bar is 25 μm.

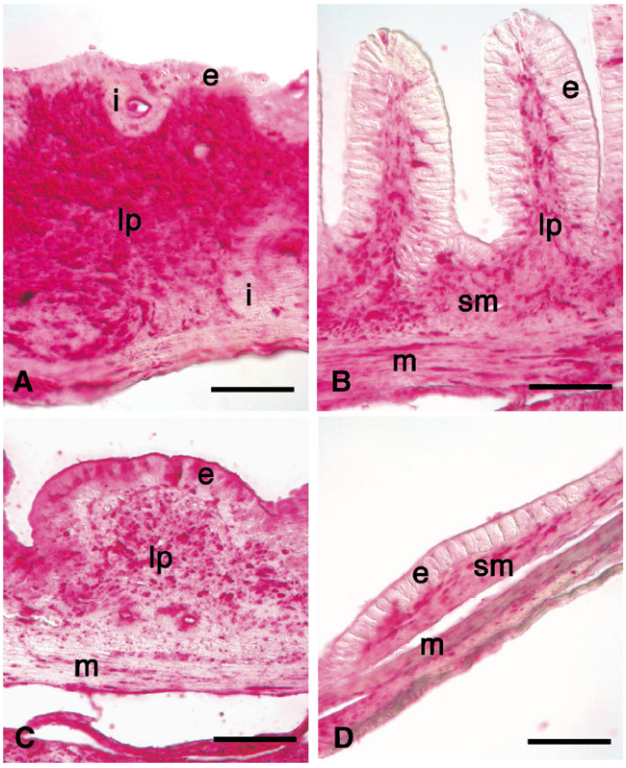

Fig. 6. Light micrographs using differential interference contrast (DIC) showing immunostaining to AQP-1 in the house sparrow lower intestine. A, cecae, B, proximal rectum, C, distal rectum and D, coprodeum. e, epithelium, i, intestinal glands, lp, lamina propria, sm, submucosa and m, muscularis. Scale bars are 50 μm.

the submucosa of the coprodeum, while no staining was evident in the epithelium (Fig. 6D). Some immunostaining was evident in the muscularis for AQP-1 (Fig. 6D).

4. Discussion

The permeability of water across the epithelium of the nephron and lower intestine of birds is too high to be accounted for by diffusion through the lipid bilayer. Large amounts of water must pass through epithelia by a different mechanism. This study identified the presence of an AQP-1 homologue in the kidney and intestinal tract of the house sparrow. AQP-1 is a membrane bound protein found in all tubule types in the renal medulla, in the glomerular podocytes, proximal tubule and the distal tubule of the renal cortex. Within the lower intestinal tract, AQP-1 was found from the cecae to the coprodeum. Its epithelial distribution was limited to the distal rectum. As AQPs serve as channels for water movement, the functional interpretation of our data suggest that AQP-1 may be involved in water movement in the kidney and the distal rectum of the lower intestine. In addition to AQP-1, other AQPs may also be involved, but their identification was beyond the scope of this study. AQP-1 is integral to water reabsorption in animal cells. Experiments with AQP-1 knockout mice demonstrate that the animals experience severe dehydration upon water deprivation due to disruption of the countercurrent multiplier system (Ma et al., 1998).

Like all birds, house sparrows have relatively inefficient kidneys that are able to produce only a slightly hyperosmotic urine. The typical urine to plasma osmolality ratio in hydrated house sparrows is 1.0 (Goldstein and Braun, 1988). The mechanism of urine concentration in the avian kidney is not entirely understood. The avian looped nephron is thought to recycle sodium chloride from the thick ascending (TAL) and into the thin descending limb (TDL) of Henle, thereby developing a concentration gradient along the length of the loop (Nishimura et al., 1989). In an earlier study on Japanese quail, both limbs of the loop of Henle were shown to be virtually impermeable to water, an unexpected observation given that the mammalian TDL is highly water permeable (Nishimura et al., 1989). However, our study in sparrows demonstrated the presence of AQP-1 in both limbs of the loop of Henle suggesting that the limbs may be water permeable. An earlier microperfusion study of Nishimura et al. (1989) demonstrated that the quail collecting duct is highly permeable to water. Our study supports this conclusion as indicated by the fact that the collecting duct immunostained for AQP-1.

To prevent excessive loss, house sparrows must reabsorb a significant amount of water and ions through the epithelium of the lower intestinal tract (Goldstein and Braun, 1988). Like the kidney, the mechanism by which birds reabsorb water in the lower intestinal tract is not clear. Both paracellular and transcellular routes have been hypothesized. We found AQP-1 present within the mucosa of the lower intestine, suggesting that it may play a role in water movement.

In birds, the amount of water and ions reabsorbed by the lower intestine varies depending on the hydration state of the animal. Studies suggest that as much as 70% of all sodium and water entering into the lower intestine may be reabsorbed in dehydrated birds (Skadhauge, 1981; Thomas, 1982). More recent studies on hydrated house sparrows demonstrated that of the fluid emptying into the cloaca, 31% of all sodium and 34% of all water was reabsorbed across the lower intestine (Goldstein and Braun, 1986, 1988). These studies did not address the specific region(s) of the intestine involved in reabsorption. Other physiological studies indicate that sodium-linked water reabsorption occurs mostly in the cecae and rectum (Thomas and Skadhauge, 1982, 1989). Our immunohistochemical data demonstrated the presence of AQP-1 in each of these areas. In addition, our RT–PCR data indicated the presence of mRNA coding for AQP-1 in each of these areas.

In birds, water moves retrogradely towards the cecae from the coprodeum, whose squamous epithelium is not conducive to water reabsorption. This orad movement allows ureteral urine to pass over the villi of the rectum whose epithelium has a high surface area and packing density that is conducive to water reabsorption (Casotti, 2001a). Coinciding with this, the present study found AQP-1 in the epithelium of the distal rectum. These findings add further evidence to the importance of retrograde peristalsis for water conservation. The present study also reports that AQP-1 although present in the submucosa of the coprodeum, is absent from the epithelium of the coprodeum. To date, no study has examined the role of the coprodeum in the reabsorption of water and ions. Studies on uptake of water and

ions have combined coprodeal and rectal segments together, leaving the contribution of the coprodeum to transepithelial water and ion uptake unknown (Skadhauge and Thomas, 1979; Thomas and Skadhauge, 1979). The absence of AQP-1 in the epithelium of the coprodeum, combined with the absence of villi in the coprodeum, suggests that it functions as a temporary storage area for ureteral urine until it can be refluxed in an orad direction toward epithelia that favor reabsorption.

At the cellular level, if AQP-1 is involved in the reabsorption of water directly from the lumen of either the kidney or the intestine it should be present at the apical membrane of the cell. With the exception of the brush border of the proximal tubule and the distal tubules, AQP-1 appears to be either distributed within the cell or basally located. Given its location, AQP-1 may play a role in either the intracellular movement of water or the movement of water across the basolateral membrane into the blood. The basal location of AQP-1 suggests that water is likely to enter the cell by a different mechanism, possibly by diffusion through the lipid bilayer or by the aid of as yet unidentified proteins. Another possibility is that the localization of AQP-1 may shift within the cell. Some mammalian AQPs, such as AQP-2, are present in intracellular vesicles and are known to shift their position within the cell (Wade et al., 1981; Nielsen and Agre, 1995). AQP-6 and AQP-8 have also been found to occur in vesicles (Yasui et al., 1999; Elkjær et al., 2001).

AQP-1 appeared in the nuclei of tubules in the renal medulla. AQP-1 was not associated with nuclei in the renal cortex or any area of the intestinal tract. The appearance of the AQP-1 reaction product in renal medulla nuclei may be the result of immunostaining of the nuclear envelope. Given that nuclei diameter is less than the section thickness (5 μm), any immunoreaction in the nuclear envelope would appear as immunostaining of the entire nucleus. These data suggest that water may move between the nuclei and the cell cytoplasm.

In addition to structures involved in osmoregulation, AQP-1 is present in the muscularis mucosa of the rectum and coprodeum. Previous research has identified AQP-1 in smooth muscle (Koyama et al., 1999) and AQP-4 in skeletal muscle in mammals (Frigeri et al., 1998). The physiological role of AQPs in muscle is unknown. Frigeri et al. (1998) proposed that AQP-4 functions to allow water diffusion across the sarcolemma to maintain osmotic equilibrium as lactate accumulates in skeletal muscle during exercise. Smooth muscle lining the avian lower intestine is in a constant state of contraction allowing for the orad movement of ureteral urine in the lower intestine (Brummermann and Braun, 1994). The smooth muscle, because of its slow rate of contraction, is unlikely to accumulate lactate, thus the functional role of AQP-1 in intestinal smooth muscle may differ from that of mammalian skeletal muscle.

In summary, this study identified and isolated a 971-bp complete cDNA of AQP-1 from the kidney and lower intestinal tract of the house sparrow *P. domesticus*. This finding is significant as the tissue distribution of AQP-1 suggests a physiological role for aquaporins in water conservation in birds. Because this AQP has been found in the lower intestine, the distribution of AQP-1 may also highlight the importance of retrograde peristalsis to water balance in birds. The high degree of homology between bacterial, plant, amphibian, mammalian and now avian aquaporins suggests an orthologous group of proteins with functional conservation.

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