Histological and histochemical study on the mesonephric kidney of *Pelophylax bedriagae* (Anura: Ranidae)

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Received: 12.07.2018  
Accepted/Published Online: 17.01.2019  
Final Version: 01.03.2019

Abstract: The purpose of this study was to determine the histological structure of the kidney of *Pelophylax bedriagae* and the distribution of hyaluronic acid (HA) in the kidney tissue. The kidneys of *P. bedriagae* are long, brown, ribbon-like structures covered with connective tissue. Nephrons, the functional units of the kidney, consist of the renal corpuscle, proximal and distal tubules, collecting tubule, and collecting duct. The renal corpuscle is composed of the glomerulus and Bowman's capsule. There is no structure similar to Henle's loop, which is found in higher vertebrates. The visceral layer of Bowman's capsule is composed of podocytes surrounding the glomerular capillaries. The parietal layer of Bowman's capsule is lined with a simple squamous epithelium. The proximal tubule is formed by cuboid epithelial cells with a brush border, and the distal tubule is covered with a simple cubic epithelium without a brush border. The collecting ducts consist of columnar or cubic cells, and they are larger than the proximal and distal tubules. Many melanomacrophage centers were observed in the kidney parenchyma. The localization of HA was determined to be in the interstitium surrounding the collecting ducts. HA probably plays a significant role in renal water handling and electrolyte balance due to its ability to retain water and bind cations.

Key words: Amphibian, mesonephros, kidney, hyaluronic acid, collecting duct

In vertebrates, the kidneys play a prominent role in the regulation of body fluid (Junqueira and Carneiro, 2006). Additionally, the kidneys are involved in many physiological processes such as the production of red blood cells, calcium metabolism, and blood pressure regulation (Epstein, 1997). The functional and structural unit of the kidney is the nephron, which is generally composed of the renal corpuscle, proximal and distal tubules, Henle's loop, and collecting tube and duct (Junqueira and Carneiro, 2006).

During the ontogenesis of vertebrates, 3 distinct forms of kidney appear: pronephros, mesonephros, and metanephros. The pronephros and mesonephros are present and functional in the life cycle of amphibians. The amphibian excretory system, pronephros in larvae and mesonephros in both larvae and adults, is important for osmoregulation and excretion (Saxén and Sariola, 1987; Möbjerg, 2000). The pronephric kidney is present in all vertebrates. However, the pronephric kidney of mammals is a nonfunctional transitory structure, which is displaced by first the mesonephros and then the metanephros. The metanephric kidney in mammals may be composed of as many as 1,000,000 nephrons. However, the pronephric kidneys of amphibians and fish consist of 1–3 nephrons (Saxén and Sariola, 1987; Ma and Jiang, 2007). The pronephric kidney is one of the typical metanephridial systems in invertebrates. Although the mesonephric kidney is more complex than the pronephric kidney, it forms a link between the metanephridial system of invertebrates and the metanephric kidney of adult amniotes (Drummond and Majumdar, 2003; Ichimura and Sakai, 2017). Therefore, understanding the amphibian kidney system is essential for understanding vertebrate kidney structure and nephron function.

Hyaluronic acid (HA) is a linear glycosaminoglycan composed of repetitive disaccharide units of glucuronic acid and N-acetylglucosamine (Toole, 2001). HA has many important functions in many groups, from bacteria to vertebrates, including humans (Almond, 2007). HA was previously considered a passive molecule, but in recent years, researchers have reported important functions of HA, including proliferation, regulation of cell–cell and cell–matrix adhesion, repair, and regeneration (Zimmerman et al., 2002; Jiang et al., 2007). Previous studies on HA have shown that HA is distributed in different parts of the body such as the skin, muscle, brain, lung, vitreous fluid of the eye, and synovial fluid, which lubricates joints of the body (Kogan et al., 2007; Necas et al., 2008; Akat et al., 2014).

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HA is a highly charged hydrophilic molecule in nature, and it is capable of absorbing 1000 times its own weight in water. Therefore, HA is essential for maintaining tissue structure and volume (Cowman and Matsuoka, 2005; Necas et al., 2008).

The current study was carried out to examine the histological and histochemical characterization and the distribution of HA in the kidney of *P. bedriagae* in order to understand its role.

The study was performed in accordance with the guidelines of the Animal Research Ethics Committee at Ege University (2018-077). Adult specimens of *P. bedriagae* (3 males and 3 females) were obtained in Izmir, Turkey (38°50’N, 23°29’E). Animals were anesthetized and then euthanized by decapitation. The kidney tissues were fixed in 4% paraformaldehyde or Bouin’s fluid and embedded in paraaffin. Deparaffinized sections were stained with hematoxylin and eosin (HE) and periodic acid–Schiff (PAS). The distribution of HA was demonstrated by using biotinylated hyaluronic acid binding protein (B-HABP). Sections were deparaffinized and washed in phosphate buffered saline (PBS) containing 2% bovine serum albumin (BSA) to suppress nonspecific binding of the antibody; sections were then incubated in B-HABP. Sections were stained with diaminobenzidine (DAB) after being washed in PBS. To determine the specificity of HA’s immunoreactivity, we used Streptomyces hyaluronidasedigested sections prior to treatment with the antibody. The sections were treated with B-HABP/HA for a positive control. Sections were examined with a Zeiss Axioscope A1 microscope attached to an Axiocam Erc 5s digital camera (Zeiss, Oberkochen, Germany).

The kidneys of *P. bedriagae* were long, brown, ribbon-like structures. The renal capsule surrounded the kidney, and the distinction between medulla and cortex was unclear. Morovvati et al. (2012) reported that the kidney of *Barbus pectoralis* (Actinopterygii: Cyprinidae) is divided into 3 parts (head, body, and caudal parts) as in other fish species, e.g., *Ctenopharyngodon idella* (Actinopterygii: Cyprinidae) (Morovvati et al., 2011), *Acipenser persicus* (Actinopterygii: Acipenseridae), and *Huso huso* (Actinopterygii: Acipenseridae) (Charmi et al., 2010). These parts were not observed in the kidney of *P. bedriagae*. Our results are in accordance with the observations of Capaldo et al. (2016) for *Triturus carnifex* (Amphibia: Urodela) and Meiberg et al. (2004) for *Geotrypetes seraphini* (Amphibia: Gymnophiona). During the evolution of vertebrates, the development of renal anatomy appears to be quite conservative when compared to the evolution of respiratory and cardiovascular systems. Major anatomical changes in vertebrate kidneys include the arrangement of Henle’s loops, division of the kidney into cortex and medullary portions, and an increase in the total number of nephrons per kidney from fish to mammals (Long and Giebisch, 1979).

The renal corpuscle is composed of Bowman's capsule and the glomerulus. Bowman's capsule was composed of 2 layers, the visceral and parietal. The visceral or internal layer surrounded the glomerular capillaries through podocytes. The parietal or outer layer consisted of a simple squamous epithelium. The space between the visceral and parietal layers was Bowman’s space, which receives glomerular filtrate (Figure 1A). The proximal tubule was formed by cuboid epithelial cells with an apical brush border, which was clearly seen with PAS staining. The distal tubule was composed of a simple cubic epithelium without a brush border (Figure 1B). There is no structure similar to Henle's loop in *P. bedriagae*. The collecting duct was formed by a cubic or columnar epithelium, and the collecting ducts were larger than the proximal and distal tubules. Melanomacrophage centers were also observed in the kidney parenchyma (Figure 1C). The localization of HA was determined to be in the kidney tissue of *P. bedriagae*. HA immunoreactivity indicated that it was located in the interstitium surrounding the collecting ducts (Figure 1D), but HA was absent around the renal corpuscle and proximal and distal tubules (Figure 1A).

Amphibians play a significant role as a study model for research on many biological processes (Feder and Burggren, 1992). Studies related to the biology of amphibians make a major contribution to many areas: morphology, histology, embryology, ecology, endocrinology, genetics, and public health. However, there have been few studies on the microanatomy of amphibian kidneys. The present study was conducted to examine the histological and histochemical characterization and distribution of HA in the kidney of the anuran amphibian *P. bedriagae*. The nephron is composed of a renal corpuscle, proximal and distal tubules, and collecting tubule and duct. The localization of HA was determined to be in the interstitium surrounding the collecting ducts.

The mesonephric kidneys of *P. bedriagae* were long, brown, ribbon-like structures. The renal capsule surrounded the kidney, and the distinction between medulla and cortex was unclear.
The glomerular capillaries lie between 2 arterioles, the afferent and efferent. Therefore, glomerular blood pressure is higher than that of other capillaries (Junqueira and Carneiro, 2006).

The proximal tubule is formed by cubic epithelial cells with an apical brush border. Krayushkina et al. (1996) reported that proximal tubules are lined by columnar epithelium with brush border in young individuals of *H. huso* and *A. persicus*. However, proximal tubules are lined with rounded squamous cells in *A. naccani* (Ojeda et al., 2003). Sodium chloride, water, and glucose are absorbed by the proximal tubules. Sodium chloride also continues to be absorbed in the distal tubule, but water absorption does not continue (Holden et al., 2013). The distal tubule is composed of a simple cubic epithelium without a brush border in the kidney of *P. bedriagae*.

Generally, each nephron is composed of the renal corpuscle, the proximal and distal tubules, Henle’s loop, and the collecting tubule and duct (Junqueira and Carneiro, 2006). However, in *P. bedriagae*, there is no structure similar to Henle’s loop as found in higher vertebrates. Many adult amphibians absorb water via the skin instead of drinking water through their mouth (Hoffman and Katz, 1999). The urinary bladder is also one of the most important osmoregulatory organs in many anurans (Suzuki et al., 2007). It is well known that *P. bedriagae* is an aquatic vertebrate. Therefore, there is probably no need to concentrate urine due to the large volume of water entering the frog through its skin.

HA immunoreactivity indicated that it was located in the interstitium surrounding the collecting ducts, but HA was absent around the renal corpuscle and proximal and distal tubules. HA is known as a natural moisturizer, and it is one of the most hydrophilic compounds in nature (Necas et al., 2008). Corroborating our results, Hansell et al. (2000) reported that HA is present almost exclusively in

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**Figure 1.** Light microscopic view of the kidney of *P. bedriagae*. A) Simple squamous epithelium of the parietal layer of Bowman’s capsule (black arrow), podocytes in the visceral layer of Bowman’s capsule (red arrow), Bowman’s space (*), glomerulus (G). B) Proximal tubule (PT), distal tubule (DT), glomerulus (G). C) Collecting duct (ellipse), melanomacrophages in the kidney parenchyma (black arrow). D) The localization of HA mainly in the interstitium surrounding the collecting ducts.
the renal medullary interstitium of rats and is not found in the cortex. Additionally, HA is a highly negatively charged molecule, and therefore it binds to cations (Plopper et al., 2013). The presence of HA in the interstitium surrounding the collecting ducts provides the passive diffusion of water from the collecting ducts to the interstitium. Therefore, HA probably has an important role in renal water handling and electrolyte balance.

In conclusion, we provide a histological and histochemical examination of the mesonephric kidney of *P. bedriagae*. The current study is the first report to reveal the distribution of HA in the amphibian kidney. Considering the presence of HA exclusively in the interstitium surrounding the collecting ducts and its ability to retain water and bind cations, HA probably plays a significant role in renal water handling and electrolyte balance.

References


