THREE-DIMENSIONAL RECONSTRUCTION OF AVIAN MEDULLARY CONE AND LATERAL RELATIONSHIPS OF LOOPS OF HENLE

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ABSTRACT

Although birds and mammals are the only vertebrates that can produce urine hyperosmotic to their plasma, the organization of the renal medulla of birds considerably differs from that of mammals. In this study, a three-dimensional model was generated from one cone in the renal medulla of a desert quail (Callipepla gambelii), and the spatial relationships between its limbs of loops of Henle and its collecting ducts were examined. Data on the spatial segregation of the collecting ducts and limbs of loops of Henle were collected. Collecting ducts formed a ring in the center, loops of Henle reaching deep into the medulla clustered within the ring, and loops ending superficially ended outside the ring. It was found that limbs of the same type did not cluster and that each loop’s pair of limbs remained near each other throughout the medullary cone. However, loops of Henle near the center of the cone, within the cluster of collecting ducts, tended to be longer than those outside. It is thought that this may have ramifications on the ability of the desert quail to concentrate its urine.

INTRODUCTION

Among vertebrates, only mammals and birds are known to produce urine at a higher concentration than their plasma, though birds’ ability to concentrate is more limited [2]. The countercurrent multiplication mechanism through which mammalian kidney nephrons concentrate their filtrate is well known [2, 3, 4]. The mammalian nephron forms what is known as a loop of Henle, travelling from renal cortex to medulla (in what is called a descending limb of Henle, or DLH) then in a hairpin turn back to the cortex (as an ascending limb of Henle, or ALH), at which it attaches to a collecting duct (or CD). The mammalian DLH is permeable to water though less so to salts, and thus the filtrate
will osmotically equilibrate with the outer interstitium. The mammalian ALH transports Na+, K+, and Cl− into the medullary interstitium, while remaining impermeable to water, thus diluting the filtrate while making the medulla hyperosmotic to plasma. Finally, the mammalian CD is capable of being permeable to water. In mammals, water thus osmotically exits the mammalian DLH and (in certain circumstances) CD (and is removed by the vasa recta capillary bed, or VR).

There are fewer data on the mechanism on urine concentration for birds [2]. Although there is evidence that a similar countercurrent mechanism occurs, there are significant morphological differences in their kidneys from mammals, both gross and microscopic. The kidney of the mammal grossly consists of a central medulla surrounded by a cortex, and may be divided into unipapillate or multipapillate pyramids at which single CDs exit the medulla [3]. In contrast, the kidney of the bird consists of many lobules each containing its own cortex and a medullary cone. Mammalian nephrons always contain a loop of Henle, but only a small percentage of avian nephrons contain a loop [1, 4].

Furthermore, mammals’ and birds’ loops of Henle differ in how they are thick- or thin-walled. Both mammalian and avian DLHs are mostly thin-walled, and ALHs are mostly thick-walled. However, mammalian loops’ hairpin turns are thin-walled (with the exception of very short nephrons), but avian loops’ turns are always thick-walled [4]. Furthermore, avian ALHs transport only Na+ and and Cl−, and thus the gradient is built from only those two ions [2, 4].

Three-dimensional reconstructions of rats’ renal medullae were constructed in a technique described by Pannabecker et al [5], and these computer models have been
useful in analyzing the structural and functional organization of the rats’ loops of Henle and collecting ducts. In this study, a similar technique was used to create a three-dimensional model of the medullary cone from a desert quail (Callipepla gambelii). This computer model was examined to verify previous observations of the avian medullary cone, and to determine whether there was any spatial segregation of loop of Henle limbs by whether they ascended or descended (e.g. in clusters or concentric circles).

**METHODS**

A medullary cone was extracted from the kidney of a euthanized desert quail (Callipepla gambelii) and transversely sectioned into 189 pieces, each 8 μm thick, onto glass slides. The cone’s sections were individually scanned at 10× magnification in digital grayscale and aligned using Amira 2.4 computer software [Visage Imaging GmbH]. Amira 2.4 was then also used to label the tubules according to their type. CDs were large, ellipsoid, and prominent, and only CDs merged into each other when viewed from cortex toward the papilla; they were labelled first. Thick limbs of Henle were smaller and more circular than the CDs but were also large and prominent, and they would always merge only once with each other; they were labelled next. When it was found, when travelling from papilla toward the cortex, that a thick limb contracted and became a very small, washer-like tubule, that tubule would be labelled as a thin limb of Henle. Finally, VSs were irregular, thin-walled spaces that often merged and split with one another, but never combined with any other tubule; they were labelled last.

Afterwards data were collected on how close the limbs of each loop of Henle were to each other. Every ten slides each loop of Henle was classified according to whether its
limbs were “close” or “separated”, where two limbs are defined to be close when their epithelia are adjacent or separated by only interstitium and/or one capillary. The loops of both types were counted at each sampled slide, along with the number of CDs in the slide (where two fusing CDs were counted as one if they shared the same lumen).

**RESULTS**

A three-dimensional model of 180 eight-micron slides was successfully created (Fig. 1). The CDs formed an incomplete ring within the cone, with ALHs, DLHs, and VRs interspersed inside, outside, and within the ring (Fig. 2). It was confirmed that the hairpin

<table>
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Fig. 1. Three-dimensional representation of desert quail medullary cone. Collecting ducts are green, thin limbs of Henle are colored red, thick limbs of Henle are yellow, and vasa recta are blue.

Table 1. Measurements of features of desert quail medullary cone. A loop of Henle is defined to be adjacent if its two limbs’ epithelia touch or if they both touch the same vasa recta.
turns of loops of Henle were always thick (Fig. 2), and that only one of the thick limbs that were part of the hairpin turn would become a thin limb.

It was observed that ALHs and DLHs were spaced throughout the entire cone, with no apparent clustering of ALH and DLH by direction of travel or epithelium thickness (Fig. 2). ALHs and DLHs of the same loop of Henle, however, tended to be adjacent to each other or separated by only a capillary (Table 1).
It was also observed that thin limbs and thick limbs forming long nephrons were
clustered much more towards the center of the cone, within the ring of CDs. Limbs out-
side the CD ring ended much more superficially.

**DISCUSSION**

The renal medulla of birds is divided into medullary cones, smaller than the renal
medulla of the unipapillate mammalian kidney. In this study, a three-dimensional model
of an avian renal medullary cone of the desert quail was generated and its structure
analyzed. In particular, the spatial relationships between pairs of limbs of Henle as well
as the collecting ducts (CD) were examined.

It was predicted beforehand that substantial physical segregation of limbs of Henle
(either by direction of travel or by epithelium thickness) would occur, either in two differ-
ent clusters or in concentric rings, and that the CDs would form a central cluster within
the medullary cone.

The data confirmed that the CDs of the medullary cone form a ring in the center of
the cone, which fused together throughout the cone to form fewer and fewer CDs, as
was shown in [2]. However, limbs of Henle of all types were mixed throughout the entire
cone, and no spatial segregation of the limbs by like type was shown. In particular, the
distances between the two limbs of each loop of Henle tended to be very small, with lit-
tle separation between the two limbs. The limbs of the rats that were studied in [5] were
not specifically examined for the same type of spatial segregation, but were indeed of-
ten separated by intervening limbs of other loops or CDs [Pannabecker, personal com-
munication]; it was proposed this difference may be due in part to the smaller diameter
of the desert quail medullary cone, giving less space for any given loop of Henle’s limbs to be separated.

However, segregation of a different sort was found, by whether nephrons ended superficially or deeply within the desert quail medullary cone. Within the cone DLHs and ALHs forming long nephrons were grouped much more towards the center of the cone; limbs outside the CD ring were shorter. This is in contrast to that of the rat renal medulla, in which it had been found that short ALHs lie within clusters of CDs [6].

It is proposed that these two differences between the organizations of the desert quail renal medullary cone and the rat renal medulla may contribute to the difference in the two species’ ability to concentrate urine. It is well known that compartmentation of structure is important to the renal medullary concentrating mechanism and affects the gradients and flow of solute concentration and water. However, determining in what way this would happen requires further investigation. In particular, methods of quantitative analysis of medullary cone three-dimensional models would be useful in the future to confirm our qualitative observations on these lateral relationships of the loops of Henle, and to develop mathematical models of the concentration mechanism of the avian kidney.

REFERENCES


Statement by Author

This thesis has been submitted in partial fulfillment of requirements for a degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Signed: Joshua Choi