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The distribution of renal hyaluronan and the expression of hyaluronan synthases during water deprivation in the Spinifex hopping mouse, *Notomys alexis*

Ray C. Bartolo *, John A. Donald

School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia, 3217

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Abstract

Hyaluronan (HA) is a glycosaminoglycan that is synthesized by a family of enzymes called hyaluronan synthases (HASs), of which there are three isoforms (HAS1, 2 and 3) in mammals. The HASs have different tissue expression patterns and function, indicating that synthesis of HA and formation of the HA matrix may be regulated by various factors. The HA matrix has an important role in renal water handling and the production of a concentrated urine. We investigated the distribution of HA and the expression of HAS1, HAS2 and HAS3 mRNAs in the kidney of the Spinifex hopping mouse, *Notomys alexis*, a native Australian desert rodent that is reported to produce the most concentrated urine of any mammal. After periods of three, seven and fourteen days of water deprivation, the distribution of renal HA changed considerably, and there was a general down-regulation of HAS mRNA expression. It is proposed that the regulation of HA synthesis by the different HAS isoforms during water deprivation in *N. alexis*, could be influenced by the molecular mass of the HA chains produced by each isoform, followed by the rate at which the individual HAS produces HA.

Keywords: Hyaluronan; Hyaluronan matrix; Hyaluronan synthase; Kidney; *Notomys alexis*; Water deprivation

1. Introduction

Hyaluronan (HA) is a member of a family of glycosaminoglycans (GAGs) commonly found in connective tissues of all vertebrates (Comper and Laurent, 1978). HA is distinct from other GAGs because it is not linked to other proteins, it is not sulphated, and is synthesized at the plasma membrane (Rogers, 1961; Prehm, 1984). Hyaluronan is a linear polysaccharide of repeating units of D-glucuronic acid and *N*-acetyl-D-glucosamine, and as the growing chain is synthesised, it is translocated to the extracellular space allowing the polymer to grow to an extremely large size in comparison to GAGs synthesised in the endoplasmic reticulum and Golgi (Comper and Laurent, 1978; Prehm, 1984; DeAngelis and Weigel, 1994).

Studies into the synthesis of HA at the plasma membrane led to the discovery and cloning of several genes from bacteria that were termed HA synthases (HASs; DeAngelis et al., 1993). Subsequently, there have been various vertebrate HASs isolated, and three mammalian isoforms have been identified: HAS1 (Itano and Kimata, 1996), HAS2 (Spicer et al., 1996) and HAS3 (Spicer et al., 1997). Studies on vertebrate HASs have shown differences in their tissue expression patterns and function, indicating that synthesis of HA may be regulated by various factors (Spicer and McDonald, 1998). Furthermore, mammalian HASs display different enzymatic properties and their ability to produce HA with varying molecular masses influences the characteristics of the HA matrix that they comprise (Itano et al., 1999; Wang et al., 1999). Spicer and McDonald (1998) have shown functional similarities and differences exist between the vertebrate HASs and have proposed that the vertebrate HAS gene family evolved by sequential gene duplication and divergence.

The extracellular HA matrix has various roles in biological systems such as a structural component in cartilage and other tissues, a lubricant in joints, forming an extracellular matrix during the inflammatory response, and the maintenance of water and protein homeostasis (Comper and Laurent, 1978; Fraser and Laurent, 1997a). Since HA is a large hydrophilic molecule, it can become closely associated with large volumes of water that

* Corresponding author. Department of Physiology, University of Otago, PO Box 913 Dunedin, New Zealand, 9054. Tel.: +64 3 479 5604; fax: +64 3 479 7323.
E-mail address: ray.bartolo@otago.ac.nz (R.C. Bartolo).

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increase the viscosity of the solution and give the solution elastic properties. The latter is an important characteristic of the kidney during peristaltic contractions of the renal pelvis (Rogers, 1961; Fraser and Laurent, 1997a). In the healthy kidney, there is very little HA in the outer medulla and cortex, but the inner medulla accumulates high concentrations of HA, and the distribution can be altered in certain clinical conditions (Hallgren et al., 1990; Göransson et al., 2004). The high levels of HA seen in the inner medulla are thought to give mechanical support to the renal tubules and blood vessels (Ginetzinsky, 1958), but more recent studies have shown that HA has an important role in renal water transport because the HA content of the kidney changes in response to various hydration states (Hansell et al., 2000; Göransson et al., 2002). Furthermore, cultured renal cells have been shown to alter the regulation of HA production when grown in media with changing osmolalities, which suggests the renal HA matrix is influenced by changes in the osmolality of the extracellular fluid and that HA has a role in renal water handling (Hansell et al., 1999).

The renal interstitial HA matrix has spongelike properties, and the interstitium of the inner medulla has been proposed to have semisolid and viscoelastic gel-like properties due to the abundance of HA, and is therefore not a free flowing aqueous compartment (Schmidt-Nielsen, 1995; Knepper et al., 2003). Contractions of the renal pelvic wall can lead to an increase in the osmolality of the luminal fluid in the descending limbs of the loops of Henle and the collecting ducts by driving the efflux of water (but not osmolytes), via aquaporins, into the medullary interstitium. The renal HA matrix has the ability to store mechanical energy from the pelvic contractions without the need to generate high hydrostatic pressures, due to its elastic properties. The HA matrix generates negative hydrostatic pressures in the interstitium after each contraction, which leads to the efflux of water molecules from the descending loops of Henle and collecting ducts into the interstitium (Schmidt-Nielsen, 1995; Knepper et al., 2003). Therefore, the effect on urine concentration is two-fold; the countercurrent mechanism of the loop of Henle is facilitated because water is drawn out of the descending loops, and water is reabsorbed from the collecting ducts before the filtrate exits the kidney.

The distribution of HA and the expression of HASs during extended periods of water deprivation, that is, when increasing urine concentration is critical to conserve water, are yet to be studied. The Spinifex hopping mouse, *Notomys alexis*, is a native Australian desert rodent that has been reported to produce the most concentrated urine of any mammal (9370 mOsmol) and can survive without drinking water (MacMillen and Lee, 1969; Weaver et al., 1994). Thus, *N. alexis* is an ideal model to examine the role of renal HA in the urinary concentrating process. This study was designed to determine if the distribution of the renal HA matrix and the expression of HAS mRNAs changes in response to water deprivation in hopping mice.

2. Materials and methods

2.1. Animals

Spinifex hopping mice, *N. alexis*, were obtained from a breeding colony at the Deakin University Animal House, Geelong, Australia. The animals were housed in rat boxes containing straw and sawdust for bedding with wire mesh lids at 21–24 °C and a 13:11 h light–dark cycle (lights on at 08:00 h). The animals received fresh tap water *ad libitum* and were fed French White millet seed.

2.2. Water deprivation experiment

The water deprivation (WD) experiments were carried out as previously described (Donald and Bartolo, 2003). For each water deprivation time point, there was a water-replete group (control) that had *ad libitum* access to water, and a water-deprived group without access to water. There were 8 animals in each group and WD was performed for three, seven and fourteen days, respectively. The mice were placed into groups one week prior to the start of the experiment and were ear-tagged to enable identification. They were housed in their groups in sand-filled glass aquaria (W100 × H40 × L50 cm) allowing room for communal sleeping burrows. The mice were weighed and fed 20 g of millet seed daily. At the end of the respective water deprivation periods, the mice were anaesthetised by halothane inhalation followed by cervical dislocation. The kidneys were dissected free, and one was frozen in liquid nitrogen and stored at −80 °C until RNA was isolated, while the second kidney was fixed overnight at 4 °C in 4% formaldehyde (pH 7.4), then stored in 70% ethanol until processing for histochemistry.

2.3. Amplification, cloning and sequencing of HA synthase cDNAs

Kidney total RNA was isolated using Trizol (Invitrogen), which utilises the single step phenol/guanidine isothiocyanate method (Chomczynski and Sacchi, 1987). The RNA concentration was determined by spectrophotometry at 260 nm. First strand cDNA was synthesised from kidney total RNA using Superscript II (Invitrogen) as per the manufacturer's protocol. All primers were designed based on *Mus musculus* sequence data obtained from GenBank (National Centre for Biotechnology, NCBI). The accession numbers for the *M. musculus* sequences are as follows: HAS1, **NM_008215**; HAS2, **NM_008216**; HAS3, **NM_008217**.

Primer sequences, annealing temperature, and the size of the predicted PCR amplicons are shown in Table 1. PCR was performed in a total volume of 20 μL with a final concentration of: 1 × PCR buffer, 0.2 mM dNTPs, 1 μM of each forward and reverse primer, 1.0 unit of Taq DNA polymerase (Scientifix),

Table 1
Forward and reverse primers used for amplifying *N. alexis* HAS1, HAS2 and HAS3 partial cDNAs, annealing temperature (AT), and the expected size of the PCR amplicons (PS)

		Primer sequence (5′–3′)	AT (°C)	PS (bp)
HAS1	Forward	ATTCCTCAGCGCACACCTAGTG	59	164
	Reverse	AGGTAAGCGGGCTCCTCTTGG		
HAS2	Forward	CCTGCGATCTCATCATCCAAAG	60	159
	Reverse	TCACAGATTGCAAACATTTCC		
HAS3	Forward	CGTTCAGTGGCACTCTGCATTG	60	170
	Reverse	CCAGCACCTCATGGAAGATGTC		