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Hyaluronan synthases and hyaluronidases in the kidney during changes in hydration status

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ABSTRACT

Hyaluronan is a large glycosaminoglycan that is abundant in the interstitium of the renal medulla/papilla. Papillary hyaluronan increases during hydration and decreases during dehydration. Due to its gel properties and ability to retain large volumes of water, hyaluronan plays a role in renal water handling by affecting the permeability characteristics of the papillary interstitium. The focus of the present investigation was the regulation of hyaluronan metabolism in the kidney, especially during variations in hydration status.
In control papillas, HAS 2 mRNA was heavily expressed and HAS 1 and 3 mRNA were weakly distributed. HYALs 1–3 mRNA were found at high expression and HYAL 4 was only weakly expressed. In hydrated animals, the diuretic response (12-fold) was followed by a 58% elevation in papillary hyaluronan and a 45% reduction in the excreted urinary hyaluronidase activity. No difference was determined in HAS 1–3 mRNA or HYAL 1, 3–4 mRNA expression, suggesting a change in activity rather than amount of protein. In dehydrated animals, antidiuresis was followed by a 22% reduction in papillary hyaluronan and a 62% elevation in excreted urinary hyaluronidase activity. Plasma vasopressin was 2.8-fold higher in dehydrated vs. hydrated rats.
In conclusion, HAS 2 appears a major contributor to the baseline levels of hyaluronan. Reduced HAS 2 gene expression and increased excreted urinary hyaluronidase activity during dehydration contribute to the reduced amount of hyaluronan and to antidiuretic response.

1. Introduction

Hyaluronan (HA, hyaluronic acid) is a major structural component of the extracellular matrix (ECM) and is found throughout the body. HA belongs to the family of glycosaminoglycans and is a linear, negatively charged high molecular weight polysaccharide that can form an entangled network at low concentrations (Laurent and Fraser, 1992). The negatively charged network attracts cations, for example Na^+ that gives HA a high osmotic activity and attracts large amounts of water through osmosis. HA interacts with cell surface receptors (i.e., CD44, RHAMM, LEC, LYVE-1) (Bono et al., 2001; Toole, 2000; Zhuo, 2000) and regulates differentiation, migration and proliferation.

HA is predominantly found in the interstitium of the renal inner papilla (medulla): in the renal cortex, the HA content is very low, only a few percent of that in the renal papilla (Wells et al., 1990; Johnsson et al., 1996; Hansell et al., 2000; Goransson et al., 2002; Rugheimer et al., 2008a). This differential distribution of HA is important for urine concentration and dilution, and as early as 1958, Ginetzinsky found evidence that the HA-degrading enzyme hyaluronidase is excreted in the urine in larger amounts during dehydration and drops to zero during hydration (Ginetzinsky, 1958). We have previously demonstrated that during acute hydration, papillary HA increases (Goransson et al., 2002; Hansell et al., 2000; Rugheimer et al., 2008a): an effect that can attenuate water reabsorption by changing the permeability characteristics of the interstitium. In contrast, papillary HA content decreases during water deprivation, indicating that the mechanisms by which HA regulates renal fluid balance differ from those of vasopressin (AVP), one of the major hormones involved in the regulation of renal fluid balance. HA favours diuresis, AVP favours retaining of water through the aquaporins.

HA is synthesised locally in the tissue by various isoforms of hyaluronan synthase (HAS). There are three homologous mammalian HA synthases, known as HAS 1–3 (Spicer et al., 1996; Weigel et al., 1997; DeAngelis, 1999). HAS 2 is the isoform that regulates its production most markedly in response to external stimuli (Jacobson et al., 2000; Li et al., 2000). An absence of HAS 2 is lethal due to abrogated normal cardiac morphogenesis and hyaluronan mediated transformation of epithelium to mesenchyme (Camenisch and McDonald, 2000). The HA synthases are found in the inner part of the plasma membrane and newly synthesised HA is extruded into the extracellular space (Prehm, 1984).

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The length of the molecule can vary, ranging from just a few disaccharides to several thousands, giving the molecule different properties, for example, HA oligomers of ~10 ± 15 disaccharide units are angiogenetic, whereas, high molecular weight HA is antiangiogenetic (West et al., 1985; Deed et al., 1997; Laurent and Fraser, 1992; Slevin et al., 1998; Lokeshwar and Selzer, 2000; Comper and Laurent, 1978).

Hyaluronidases (HYAL) are a family of enzymes involved in the degradation of HA. There are six known human isoforms of HYAL, HYAL 1 and HYAL 2 are found in most tissues and are thought to degrade HA locally. HYAL 1 is predominant in human plasma (Frost et al., 1997).

Renomedullary interstitial cells (RMIC) are one of the major contributors of HA in the renal papilla. RMICs in culture produce less HA under hyperosmotic conditions than under iso- and hypo-osmotic conditions (Goransson et al., 2001; Hansell et al., 1999), supporting *in vivo* observations that HA content decreases during dehydration and increases during hydration (Hansell et al., 2000; Goransson et al., 2002). Despite observations that the renal HA content changes under normal physiological conditions depending on the hydration status, the mechanisms regulating HA content under these conditions are unclear. The aim of the present study was to examine changes in the mRNA levels of HAS and HYAL and in the levels of HA and urinary hyaluronidase activity during different states of body hydration i.e. 2 h hydration or 24 h dehydration.

2. Results

2.1. Control animals

HA was heterogeneously distributed in all kidneys, i.e. the cortical levels (23 ± 4 µg g^{-1} dry weight) were only a few percent of those in the papilla (1203 ± 151 µg g^{-1} dry weight). The low levels of hyaluronan in the cortex were unchanged during the different states of hydration: these values will not be discussed further. HAS 2 transcription was large in the papilla and HAS 1 and 3 were low (Fig. 1). HYALs 1–3 were highly expressed in the papilla and HYAL 4 had little expression (Fig. 2). Urine flow rate was 4.3 ± 0.4 µl min^{-1}, urine osmolality 1310 ± 218 mOsm kg^{-1} H_2O (Table 1). The urinary hyaluronidase activity (Fig. 3) was 3.97 ± 0.55 RI μl^{-1} and the excreted hyaluronidase activity was 17.61 ± 1.07 RI min^{-1}. The plasma vasopressin level was 13.1 ± 1.7 pg ml^{-1} (Table 1).

2.2. Hydrated animals (2 h hydration)

Hydration produced a 12-fold increase in diuresis ($p<0.05$) and the urine osmolality fell to 201 ± 39 mOsm kg^{-1} H_2O ($p<0.05$) (Table 1). Papillary HA content was 58% higher in hydrated animals (1904 ± 200 µg g^{-1} dry weight) than in control animals (1203 ± 151 µg g^{-1} dry weight: $p<0.05$: Fig. 4) without significant change in the transcription of either HAS 1–3 (Fig. 1). No change occurred in transcription of HYALs 1 or 4, but expression of HYAL 2 was elevated ($p<0.05$: Fig. 2). HYAL 3 did not reach a significant elevation but a similar trend as with HYAL 2 was seen (Fig. 2). The urinary hyaluronidase activity was 0.17 ± 0.05 RI μl^{-1} and the excreted hyaluronidase activity was 9.65 ± 1.79 RI min^{-1} (Fig. 3). The plasma vasopressin level was 10.1 ± 1.6 pg ml^{-1} (Table 1).

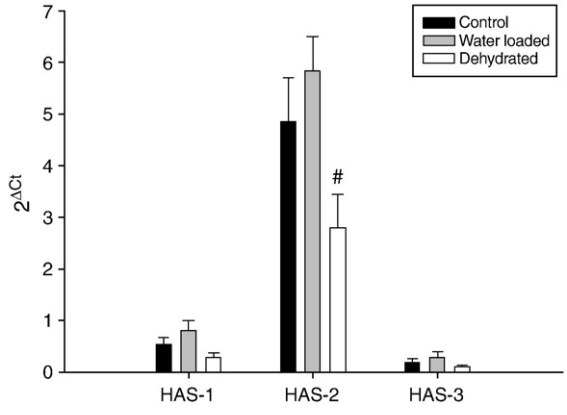

Fig. 1. Gene expression of HAS 1–3 in the renal papilla during variation in hydration status. # denotes $p<0.05$ vs. water loaded (hydrated) animals.

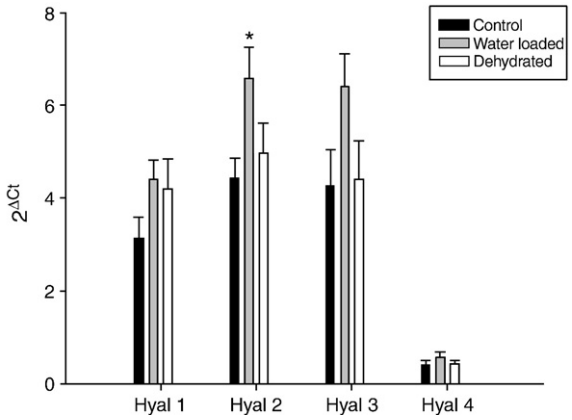

Fig. 2. Gene expression of HYAL 1–4 in the renal papilla during variation in hydration status. * denotes $p<0.05$ vs. control animals.

2.3. Dehydrated animals (24 h water deprivation)

The dehydrated animals had lower urine flow rates and higher systemic hematocrit than control animals ($p<0.05$: Table 1). Although urine osmolality had higher numerical values than control animals, it did not reach statistical significance. The papillary hyaluronan content was reduced (942 ± 101 µg g^{-1} dry weight) compared to the control and hydrated animals ($p<0.05$: Fig. 4). The gene expression of HAS 2 was lower than the hydrated animals but HAS 1 and 3 were similar between groups (Fig. 1). The expressions of HYAL 1–4 were unaffected (Fig. 2). The urinary hyaluronidase activity was 11.37 ± 0.35 RI μl^{-1} and the excreted hyaluronidase activity was 28.57 ± 2.46 RI min^{-1} (Fig. 3). The plasma vasopressin level was 28.4 ± 4.2 pg ml^{-1} which was 2.8-fold higher ($p<0.05$) than that in hydrated rats (Table 1).

3. Discussion

The present study sought to determine if changes in the transcript level of hyaluronan synthases and hyaluronidases could explain the changes observed in papillary interstitial hyaluronan during variations in hydration status. The rapid (within 2 h) elevation in papillary hyaluronan, which is evident after 2 h of hydration, did not primarily involve a change in gene

Table 1
Mean arterial blood pressure (MAP), urine osmolality, urine flow rate, body weight, kidney weight, hematocrit and plasma vasopressin in control rats, rats subjected to 2 h of hydration and rats dehydrated for 24 h.

	Time control, euvolaemia ($n=10$)	Hydrated, 2 h ($n=8$)	Dehydrated, 24 h ($n=9$)
Mean arterial blood pressure (mm Hg)	118 ± 1	110 ± 4	104 ± 2[a]
Urine osmolality (mOsm kg^{-1} H_2O)	1310 ± 218	201 ± 39[a]	1558 ± 214
Urine flow rate (µl min^{-1})	4.3 ± 0.4	66.3 ± 7.5[a]	3.0 ± 0.4[a]
Body weight (g)	294 ± 10	301 ± 11	254 ± 6
Kidney weight (g)	1.18 ± 0.03	1.14 ± 0.02	1.08 ± 0.03
Hematocrit (%)	40 ± 1	41 ± 1	43 ± 0.4[a]
Plasma vasopressin (pg ml^{-1})	13.1 ± 1.7	10.1 ± 1.6	28.4 ± 4.2[a]

Values are means ± 1 SEM.
[a] $p<0.05$ vs. corresponding parameter in control animals.