Kidney International, Vol. 50 (1996), pp. 1191–1194

Effect of water diuresis and water restriction on expression of HSPs-27, -60 and -70 in rat kidney

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Effect of water diuresis and water restriction on expression of HSPs-27, -60 and -70 in rat kidney. Expression of HSP-27, HSP-60 and HSP-70 was estimated in the cortex, outer medulla and inner medulla (papilla) of rats undergoing water diuresis or water restriction for two days. The mRNAs for HSP-27 and HSP-60 in renal papilla were two- to threefold greater in rats during water restriction than in those excreting a dilute urine, but levels of mRNA for HSP-70 were not reduced by water diuresis and Western analysis for HSP-70 protein showed no difference between water-loaded and water-restricted animals.

Heat shock proteins (HSPs) are highly conserved proteins synthesized by organisms in response to the stress of heat as well as by exposure to other stresses such as heavy metals, hypertonicity, anoxia, infection, and a variety of toxins. In particular, HSP-27, HSP-60 and HSP-70 are thought to protect cellular proteins from denaturation and to enhance the proteolysis of unfolded or abnormally folded proteins [1–3]. Because these HSPs might play a role in protecting tubular epithelial cells during adaptation of the renal medulla and papilla to high osmolarity, we studied their expression in the rat kidney during water diuresis and water restriction.

Methods

Animals

Two groups of 12 male rats (Sprague-Dawley, 290 to 310 g) were maintained on Purina Lab Chow and housed in metabolism cages that permitted quantitative collection of all urine. The water-loaded group was given 5% dextrose/water as drinking water for three days. A second group was restricted to an intake of 10 ml/day of water for two days. At the end of this time, the rats were anesthetized with 100 mg/kg of Inactin®, given intraperitoneally, the kidneys removed, and the cortex, medulla and papilla quickly dissected from each kidney. The papillae obtained from four animals were combined to prepare poly A^+ RNA. Urinary osmolarity and the concentrations of sodium, potassium and creatinine in urine were measured in both groups.

In a separate study in which HSP-70 protein was measured in the kidneys, three groups of six rats were studied. In Group I (water diuresis), 5% dextrose in water was substituted for drinking water and food was restricted to 5 g of Purina Lab Chow per day in order to encourage the intake of water. In Group II (water-restricted), food was also restricted to 5 g of Purina Lab Chow daily, and water intake was limited to 10 ml per day. In Group III (controls), food was restricted but water was allowed *ad libitum*. Animals were sacrificed on the third day. The right kidney was removed, cortex, outer medulla and papilla dissected, quickly frozen in liquid nitrogen and stored until assayed for HSP-70 protein.

RNA preparation and Northern analysis

Total RNA from kidney tissue was isolated by the guanidinium isothiocyanate/CsCl method [4], and Poly A^+ was selected in Oligo-dT columns. A total of 3.5 μg (papilla) and 5 μg (cortex and medulla) of Poly A^+ were separated in 1% agarose cells containing 0.2 M formaldehyde [5]. The RNA was transferred from the gel to nylon membranes (Gene Screen Dupont, NEN Research Pro., Wilmington, DE, USA) in 20 × SSC (3 M sodium chloride/0.3 M sodium citrate). RNA was cross-linked to the membrane by UV light at 1200 microjoules on a Stratalinker apparatus (Stratagene Co., La Jolla, CA, USA). Membranes were hybridized at 55°C with ^{32}P-labeled cDNA for heat shock proteins: HSP-70, HSP-60 and HSP-27 were radiolabeled by random primer extension [6]. GAPDH cDNA was used as an internal control to assure even loading [7]. After hybridization, the filters were washed in 0.5 × SSC/1% SDS at 55°C. Membranes were exposed to XAR-2 film (Kodak) for autoradiography.

Western analysis for HSP 70 protein

Samples from cortex, medulla and papilla were weighed and homogenized on ice in a solution containing 250 mM sucrose, 50 mM Tris, 1 mM PMSF, pH 7.4 (20 mg tissue/ml). Homogenates were centrifuged for 20 minutes at 12,000 × g at 4°C and the supernatant collected and protein content determined (BioRad). Thirty micrograms of protein was boiled for five minutes in the presence of S-mercaptoethanol and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were electrophoretically transferred to Immobilon-P membranes (Millipore), blocked for 30 minutes in PBS (150 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4) + 4% milk, rinsed briefly in PBS, and exposed to monoclonal anti-HSP-70 (clone 7 to 10, MA 3 to 001, Affinity Bioreagents) in PBS + 4% milk (1:500) overnight at 4°C. This antibody recognizes several members of the HSP-70 family including the inducible HSP-72

Received for publication December 26, 1995
and in revised form May 25, 1996
Accepted for publication May 25, 1996

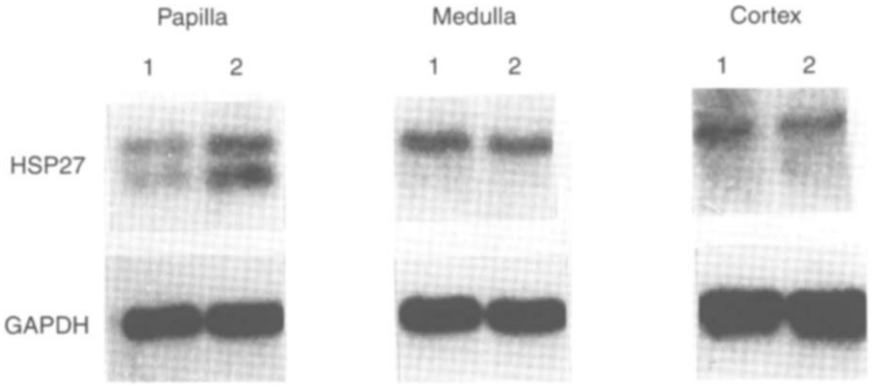

Fig. 1. *Northern analysis of mRNA from the renal papilla, medulla and cortex of rats undergoing either water diuresis (1) or water restriction (2).* Nitrocellulose blots were probed sequentially with ^{32}P-labeled HSP-27 (upper panel) and GAPDH (to demonstrate equality of loading). There is a two-fold increase in the mRNA for HSP-27 in the renal papilla following water restriction (quantitated using the ImageQuant software). Each lane represents the combined RNA from four animals.

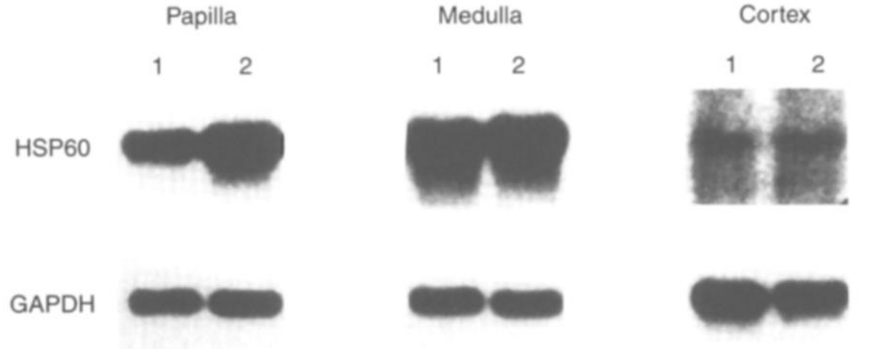

Fig. 2. *mRNA from the renal papilla, medulla and cortex of rats undergoing either water diuresis (1) or water restriction (2) reveals a three-fold increase in the message for HSP-60 in the papilla following water restriction.* Each lane represents the combined RNA from four animals.

following heat shock, as well as the constitutive HSP-70. Blots were then washed sequentially with PBS + 4% milk and PBS and exposed to horseradish peroxidase-linked rabbit anti-rat immunoglobulin (Boehringer Mannheim Biochemicals) for one hour prior to detection of proteins using a chemiluminescence system (ECL, Amersham International).

Results

Urinary volume and osmolality during water restriction and water diuresis

In 12 rats limited to a water intake of 10 ml/day, the volume of urine excreted during the second day of water restriction averaged 9.8 ± 0.1 ml/day (mean ± SE), with an osmolality of 2142 ± 126 mOsm/kg. In contrast, after two days of water diuresis, urinary volume was ten times greater (99 ± 5 ml/day) and the urinary concentration of solutes correspondingly dilute (173 ± 17 mOsm/kg).

Similar results were obtained in a second set of experiments in which 6 water-loaded rats excreted urine with an average osmolality of 117 ± 12 mOsm/kg, compared with a Uosm of 1840 ± 163 in a group of six rats allowed to drink only 10 ml of water daily.

Laboratory rats on a free diet habitually excrete a concentrated urine. As expected, control rats allowed to drink *ad libitum* excreted a urine with a concentration (1820 ± 132 mOsm/kg) comparable to that seen when water intake was restricted to 10 ml/day.

mRNA for HSPs-27, -60 and -70

In the renal cortex and medulla of rats in both water loaded and water restricted rats, a single band for HSP-27 was observed. In the papilla, however, two bands were identified, at approximately 1.0 and 1.2 kb, suggesting either two species of HSP-27 in this region or alternate splicing of the message. Following water restriction there was a twofold increase in both messages of HSP-27 in the papilla, but no change in cortex or outer medulla, when compared with the kidneys of rats undergoing water diuresis (Fig. 1).

Similarly, the level of mRNA for HSP-60 was threefold greater in the renal papilla of water-restricted rats than in those excreting a dilute urine, while its levels in outer medulla and cortex were not affected by water restriction of diuresis (Fig. 2).

Papillary (as well as medullary) levels of mRNA for HSP-70, on the other hand, were not affected by water diuresis or water restriction. HSP-70 mRNA was consistently higher in the renal papilla than in the outer medulla (Fig. 3) as described by others [8–10].

HSP-70 protein expression in kidney

Neither water loading nor water restriction induced significant changes in the expression of the HSP-70 protein in any region of the kidney (Table 1 and Fig. 4), in accordance with the results seen on Northern analysis.

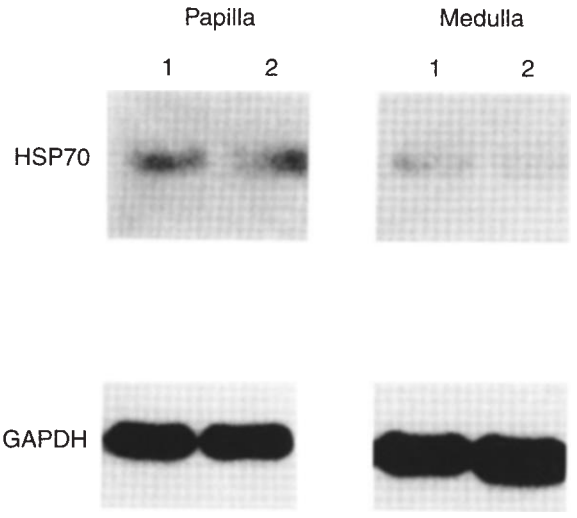

Fig. 3. *mRNA from the renal medulla and cortex of rats undergoing either water diuresis (1) or water restriction (2) reveals no change in the message for HSP-70.* Each lane represents the combined RNA from four animals.

Table 1. HSP-70 protein in kidney and Uosm after water loading and water restriction

	HSP-70 protein			U_{Osm}
	Cortex	Outer medulla	Papilla	mOsm/kg
Control	4.96 ± 0.45	5.90 ± 1.28	5.56 ± 1.09	1820 ± 132[a]
Water loaded	5.60 ± 0.48	4.61 ± 0.53	6.30 ± 1.42	101 ± 10
Water restricted	6.04 ± 0.48	5.9 ± 0.14	7.72 ± 1.57	1943 ± 124[a]

Total protein was separated by SDS-PAGE and blotted with anti-HSP-70. Autoradiographic quantification of HSP-70 was performed using the ImageQuant software. HSP-70 levels are expressed as arbitrary units/mg protein ± SEM ($N = 3$ for each value). U_{Osm} was measured on the third day of the regimen.

[a] $P < 0.001$ vs. water loaded

Discussion

The renal medulla (especially the inner medulla, or papilla) is the only place in the body where cells are regularly subject to large changes in osmotic pressure. In the course of passing from water diuresis to maximal renal water retention, the ambient osmotic pressure at the tip of the rat renal papilla may fluctuate from a level isotonic with plasma to 7 times that value, associated with an increase in the concentration of sodium in extracellular fluid of 2 to 3 times, and much larger changes in the concentration of urea in extracellular and intracellular fluids. The resultant changes in intracellular volume are blunted by changes in the uptake and synthesis of organic osmolytes [11]. Nevertheless, such sudden changes in intracellular water content must pose a risk to the correct folding, processing, localization, and in some cases complexing of cellular proteins with other polypeptides that must take place in order to carry out their biological functions. In all cells of the body, there seems to be a "chaperoning" activity to maintain proteins in an unfolded state, yet prevent undesirable interactions. This need can only be intensified in cells like those of the renal medulla and papilla, which are subject to frequent wide swings in the osmotic pressure of their bathing fluids. Many members of the diverse family of heat shock proteins, conserved from bacteria to man throughout evolution, are thought to act as chaperones which associate with newly-synthesized proteins, preventing them from misfolding and allowing them to cross biological membranes and fold properly. Under conditions of stress, chaperones protect other proteins from denaturation or, if damage has occurred, disaggregate and allow them to refold back to an active form [12].

Members of the most carefully studied family of heat shock proteins, HSP-70, appear to be mobile within the cell, moving from cytoplasm to nucleus shortly after heat shock and then returning to the cytoplasm upon recovery [1]. HSP-70 gene products are found also in specialized cell organelles such as the endoplasmic reticulum and mitochondria [12]. The HSP-60 family of proteins, which bind denatured proteins, are localized to mitochondria, where they are thought to play a global role in polypeptide chain folding [12]. HSP-27 protein is a major target of phosphorylation upon cell stimulation with a variety of agents, including heat and ischemia [13]. It has been suggested to have a phosphorylation function at the level of actin filaments [14] and to be involved in the development of resistance by cancer cells to chemotherapeutic agents [15]. In a continuous cell line derived from human breast cancer [16], HSP-27 proteins are said to increase in response to osmotic stress, although there is no published information about the behavior of HSP-27 in osmotically stressed renal cells.

Hyperosmotic stress was also found by Cohen, Wasserman and Gullans [17] to increase the expression of mRNA for HSP-70 in cultured Madin-Darby canine kidney (MDCK) cells, which retain many characteristics of distal tubular cells in intact mammalian kidneys. These authors therefore postulated that HSP-70 might play a role in intact kidneys to stabilize proteins in the face of the elevated and potentially denaturing intracellular ionic concentrations that accompany sudden changes in the medullary osmotic environment.

The present experiments indicate that the messages for HSP-27 and HSP-60 are higher in rat renal papilla when the kidney is excreting a concentrated urine and renal tubular cells are exposed to a hyperosmotic environment than during water diuresis, when medullary tonicity falls to approach that of the circulating blood. It is not yet clear whether or how these changes, presumably paralleled by changes in production of the relevant heat shock proteins, protect renal papillary cells against hyperosmotic stress. Contrary to our expectations, neither the HSP-70 message nor its protein product differed significantly in renal papillae of rats excreting a concentrated urine from those of rats induced to excrete a dilute urine for two days. It is possible that more prolonged water diuresis might have attenuated expression of HSP-70 in renal cells. It also seems possible that the rapid accumulation or release of organic osmolytes by renal papillary cells may reduce the HSP-70 response to changes in tonicity, as suggested by Sheikh-Hamad et al in MDCK cells [18].

In summary, mRNAs for HSP-27 and HSP-60, were increased in the renal papillae of rats excreting a dilute urine, as compared with rats excreting a concentrated urine. Neither the message (mRNA) nor the protein product of HSP-70 in renal papilla, outer medulla, or cortex, was altered by water loading or water restriction. These data are consistent with a function for HSPs in protecting against osmotic stress in the renal papilla but do not support an adaptive role in this regard for HSP-70.

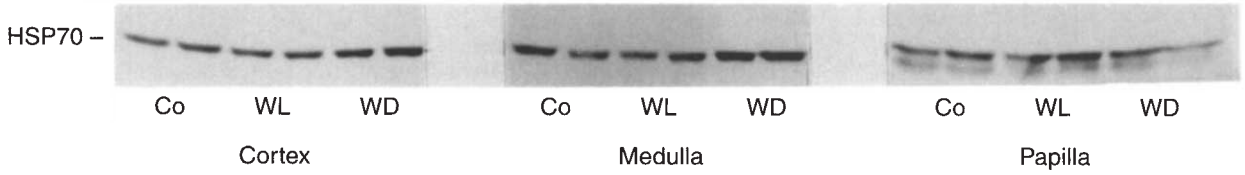

Fig. 4. *Whole cell lysates from the renal papilla, medulla and cortex of rats undergoing either water loading (WL) or water deprivation (WD) were compared to control rats (allowed to drink ad libitum) for level of expression of the HSP-70 protein.* Neither water loading nor water deprivation appreciably altered HSP expression. ($N = 2$ for each condition).

Acknowledgments

These experiments were supported in part by grants from the American Heart Association and the National Institutes of Health (DK-18078) to F.H. Epstein.

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References

1. SCHLESINGER MJ: Heat shock proteins: The search for functions. *J Cell Biol* 103:321–325, 1986
2. ANG D, LIBEREK K, SKOWYRA D, ZYLIEZ M, GEORGOPOULOS AC: Biological role and regulation of the universally conserved heat shock proteins. *J Biol Chem* 266:24233–24236, 1991
3. ANATHAN J, GOLDBERG AL, VOELLMY R: Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. *Science* 232:522–524, 1986
4. CANTLEY LG, ZHOU Z-M, CUNHA MJ, EPSTEIN J, CANTLEY LC: Ouabain-resistant transfectants of the murine ouabain resistance gene contain mutations in the α-subunit of the Na,K-ATPase. *J Biol Chem* 267:17271–17278, 1992
5. JOANNIDIS M, SPOKES K, NAKAMURA T, FALETTO D, CANTLEY LG: Regional expression of hepatocyte growth factor/c-met in experimental renal hypertrophy and hyperplasia. *Am J Physiol* 267:F231–F236, 1994
6. HINCKNEY E, BRANDON SE, SADIS S, SMALE G, WEBER LA: Molecular cloning of sequences encoding the human heat-shock proteins and their expression during hyperthermia. *Gene* 43:147–154, 1986
7. FORT P, MARTY L, PIECHACZYK M: Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Nucl Acids Res* 13:1431–1432, 1985
8. EMAMI A, SCHWARTZ JH, BORKAN SC: Transient ischemia or heat stress induces a cytoprotectant protein in rat kidney. *Am J Physiol* 265:F479–F485, 1991
9. JOANNIDIS M, CANTLEY LG, SPOKES K, MEDINA R, PULLMAN J, ROSEN S, EPSTEIN FH: Induction of heat-shock proteins does not prevent renal tubular injury following ischemia. *Kidney Int* 47:1752–1759, 1995
10. COWLEY BD JR, GUDAPATY S: Temporal alterations in regional gene expression after nephrotoxic renal injury. *J Lab Clin Med* 125:187–199, 1995
11. GARCIA-PEREZ A, BURG MB: Renal medullary osmolytes. *Physiol Rev* 71:1081–1115, 1991
12. ELLIS RJ, VAN DER VIES SM: Molecular chaperones. *Ann Rev Biochem* 60:321–347, 1991
13. ANDRES J, SHARMA HS, KNOLL R: Expression of heat shock proteins in the normal and stunned porcine myocardium. *Cardiovasc Res* 27:1421–1429, 1993
14. HUOT J, LAMBERT H, LAVOIE JN, GUIMOND A, HOULE F, LANDRY J: Characterization of 45-kDa/54-kDa HSP 27 kinase, a stress-sensitive kinase which may activate the phosphorylation-dependent protective function of mammalian 27-kDa heat-shock protein HSP 27. *Eur J Biochem* 227:416–427, 1995
15. HUOT J, ROY G, LAMBERT H, CHRETIEN P, LANDRY J: Increased survival after treatments with anti-cancer agents of Chinese hamster cells expression the human Mr 27,000 heat shock protein. *Cancer Res* 51:5245–5252, 1991
16. KATO M, BRIJLALL D, ADLER SA, KATO S, HERZ F: Effect of hyperosmolality on alkaline phosphatase and stress-response protein 27 of MCF-7 breast cancer cells. *Breast Cancer Res Treat* 23:241–249, 1992
17. COHEN DM, WASSERMAN JC, GULLANS SR: Immediate early gene and HSP-70 expression in hyperosmotic stress in MDCK cells. *Am J Physiol* 261:C594–C601, 1991
18. SHEIKH-HAMAD D, GARCIA-PEREZ A, FERRARIS JD, PETERS EM, BURG MB: Induction of gene expression by heat shock *versus* osmotic stress. *Am J Physiol* 267:F28–F34, 1994