Urol Res (1999) 27:306–311
© Springer-Verlag 1999

ORIGINAL PAPER

**Ziya Akçetin · Reinhard Pregla · Dorothea Darmer
Hans Heynemann · Johannes Haerting
Hans-Jürgen Brömme · Jürgen Holtz**

Differential expression of heat shock proteins 70-1 and 70-2 mRNA after ischemia-reperfusion injury of rat kidney

Received: 16 November 1998 / Accepted: 11 March 1999

Abstract Ischemia-reperfusion injury in the kidney is known to cause induction of the inducible form of the 70 kDa heat shock protein HSP70i (or HSP72). However, knowledge of the expressional regulation of the two coding genes for HSP70i – *HSP70-1* gene and *HSP70-2* gene – is very limited. We investigated the time course of HSP70-1 and -2 mRNA expression and its relation to cellular ATP levels in the renal cortex after different periods of unilateral warm renal ischemia (10–60 min) and reperfusion (up to 60 min) in 10-week-old male Wistar rats. Immediately after ischemia there was a significant induction of both *HSP70i* genes. While HSP70-1 expression constantly increased (up to 4-fold) during reperfusion, even to a higher extent with prolongation of ischemia, HSP70-2 mRNA – which was generally expressed at a far lower level than HSP70-1 mRNA – was strongly induced (3-fold) during reperfusion only after brief periods (10 min) of ischemia. Cellular ATP levels rapidly dropped to 5% with ischemia and the pattern of recovery during reperfusion significantly depended on the duration of the ischemic period, thus showing a good relation with the heat shock (protein) gene expression. We conclude that *HSP70-2* is the more sensitive gene with a lower activation threshold by mild injury, while the *HSP70-1* gene mediates the major response of heat shock protein induction after severe injury.

Key words Ischemia-reperfusion · Heat shock · HSP70 · Kidney · Apoptosis

Dedicated to the memory of the late Prof. Giyaseddin Adil Korkud (1.4.1913–29.12.1998)

Z. Akçetin · R. Pregla · H. Heynemann
Department of Urology
of the University of Halle-Wittenberg, Halle, Germany

D. Darmer · H.-J. Brömme · J. Holtz
Department of Pathophysiology
of the University of Halle-Wittenberg, Halle, Germany

J. Haerting
Department of Biometrics
of the University of Halle-Wittenberg, Halle, Germany

Z. Akçetin (✉)
Klinik und Poliklinik für Urologie
der Martin-Luther-Universität, Magdeburger Strasse 16,
D-06097 Halle, Germany

Introduction

Organ response to injury is modulated by the expression of genes which are necessary for cell and organ survival during and after the insult. In this context expression of heat shock proteins (HSP) – a universal stress response remarkably conserved throughout evolution – is extremely important and probably the best-characterized system of cellular defense. HSP belong to a diverse family of inducible and constitutive stress proteins [13, 17, 18]. In mammalian cells, the inducible 70-kDa HSP (HSP72 or HSP70i) is the most abundant HSP [31] and is the HSP most closely linked to cytoprotection [28] from a variety of dangerous events such as thermal injury or ischemia-reperfusion. In rat and human, HSP72 is encoded by two genes that differ within each species only in the 3′-untranslated region [33]. This similarity of HSP70-1 and -2, encoding identical proteins, is exceptional. So far, only some preliminary data indicate that HSP70-1 and -2 can be expressed discordantly in humans [24] and rats [33]. The fact that two genes encode identical proteins may be due to the need to express the same protein under different circumstances in a different pattern [33].

We therefore studied HSP70-1&2 "sum" mRNA expression in renal cortex after short (10 min), moderate (40 min), long (60 min) and extremely long (120 and 180 min) periods of ischemia and during subsequent reperfusion (5–60 min), and compared these results with HSP70-2 "only" mRNA expression. Since the ATP level is supposed to be an important trigger of the heat shock response [3, 15], we also determined the renal ATP content in the same material.

Material and methods

Animal preparation and experimental set-up

Unilateral warm renal ischemia was conducted on male Wistar rats (10 weeks old) for 10, 40 60, 120 and 180 min under pentobarbital anesthesia (100 mg/kg body weight i.p.). Only data from rats with 10–60 min of ischemia and subsequent reperfusion are reported here. After reperfusion times of 0, 5, 10, 30 and 60 min ($n = 10$ for each reperfusion group), left nephrectomy was performed. All surgical procedures were performed at 0900–1100 hrs and body temperature was kept constant at 37°C throughout. Contralateral kidneys of experimental rats and kidneys from rats exposed to anesthesia but not to ischemia-reperfusion, served as controls. During the animal experiments, Principles of Laboratory Animal Care were followed, and the current version of the "German Law on the Protection of Animals" was applied.

Analysis of high-energy adenine nucleotides

Part of the kidney was frozen in situ in liquid nitrogen for quantitative determination of ATP (Bioluminescence Assay Kit CLS II, Boehringer Mannheim, Germany). These data have been reported elsewhere in the context of the analysis of renal antioxidative capacity [1]. The remaining part of some kidneys was used for mRNA analysis (4–10 per group).

RNA isolation

All experimental steps were performed under RNAse-free conditions. Total RNA was isolated from renal tissue by mechanical crushing in liquid nitrogen, homogenization in guanidinium isothiocyanate solution and centrifugation through a cesium chloride cushion [4]. Concentration of RNA was calculated from the A_{260} values. Quality of isolated RNA was assessed by agarose gel electrophoresis.

Reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA (each RT reaction containing 250 ng) was reverse transcribed in a thermocycler (Trioblock, Biometra, Göttingen, Germany) using SuperScript Plus II RNAse H^- Reverse Transcriptase (GIBCO-BRL, Eggenstein, Germany). One millimole of each dNTP (Pharmacia, Freiburg, Germany), 2.5 U RNasin (GIBCO-BRL, Eggenstein, Germany), 0.5 mg of random hexamer oligonucleotide primers and 15 U of reverse transcriptase were added to "1× reverse transcription buffer" in a total final volume of 20 µl. The reaction was incubated at 37°C for 10 min, then for 20 min at 42°C, stopped by heating at 95°C for 10 min and finally chilled on ice. This first-strand cDNA reaction was used as a template for PCR amplification containing the following components: 1× PCR buffer (complete); 12 µM each dNTP; 5 pmol each specific primer (GIBCO-BRL, Eggenstein, Germany); 2 U *Taq* DNA polymerase (Pharmacia, Freiburg, Germany). In a thermocycler (PE 9600, Perkin Elmer), amplification cycles were performed after an initial denaturation of 2 min at 95°C: 30 s denaturation at 94°C, 30 s primer annealing, 30 s extension at 72°C. Reactions with equal amounts of not-reverse-transcribed RNA were included to control for amplification of contaminating genomic DNA. This is necessary because HSP70 genes are intronless. To determine the cycle numbers for PCR products remaining in a linear phase of amplification, the PCR products of each gene were sampled at 20, 25, 30, 35 or 40 cycles (95°C for 1 min, annealing temperature for 1.5 min and 72°C for 1.5 min). After amplification, 20 µl of the PCR products were separated by 1% agarose gel electrophoresis and photographed.

Primers

Three pairs of primers specific for HSP70-1 and -2 (together), HSP70-2 and glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) were designed and synthesized (GIBCO BRL-Life Technologies) according to their sequences [18, 33]. Primer sequences and annealing temperatures (AT) were as follows:

HSP 70-1 and -2
Reverse 5′-CGCTGCGAGTCGTTGAAGTAG-3′
Forward 5′-GTCGGACATGAAGCACTGGC-3′
(AT: 62°C)

HSP 70-2
Reverse 5′-AGATCACACCTGGAGCGCC-3′
Forward 5′-GCTACAAGGCGGAGGACG-3′
(AT: 62°C)

GAP-DH
Reverse 5′-TGACCTTGCCCACAGCCTTG-3′
Forward 5′-CATCACCATCTTCCAGGAGCG-3′
(AT: 65°C)

PCR fragment elution and DNA sequencing

To verify the amplified fragments we checked not only the expected size but also the nucleotide sequences of PCR fragments after elution from the agarose gel (with a dialysis membrane) using the Dye Terminator Cycle Sequencing Ready Reactions (Perkin Elmer, Langen, Germany) and ABI automated sequencer (Applied Biosystems, Weiterstadt, Germany).

Data

Semiquantitative RT-PCR was evaluated by scanning of polaroid negatives of the gel images using a laser densitometer and a computer-based imaging system (Molecular Dynamics, Krefeld, Germany). All mRNA levels (relative units normalized to GAP-DH) were expressed as a densitometric ratio to GAP-DH mRNA and are presented relative to this ratio in the contralateral kidney. The values in all figures are given as the mean ± SEM. Statistical significance was evaluated for mRNA studies by analysis of variance, followed by individual comparisons as indicated and by the unpaired Student's *t*-test for ATP values.

Results

HSP70-1&2 mRNA

At the end of all three ischemic periods (10, 40 and 60 min) and also after 5 min of reperfusion there was no significant change in the expression levels of HSP70-1&2 mRNA compared with controls (Fig. 1). Kidneys exposed to 10 min of ischemia had only transient fluctuations in HSP70-1&2 mRNA during reperfusion (Fig. 1). However, HSP70-1&2 mRNA increased almost 3-fold during reperfusion (Fig. 1) after 40 and 60 min of ischemia. After longer periods of ischemia (120 and 180 min), reperfusion did not induce further augmentation in HSP levels (data not shown).

HSP70-2 mRNA

At the end of the ischemic period, there was no significant change in the expression level of HSP70-2 mRNA

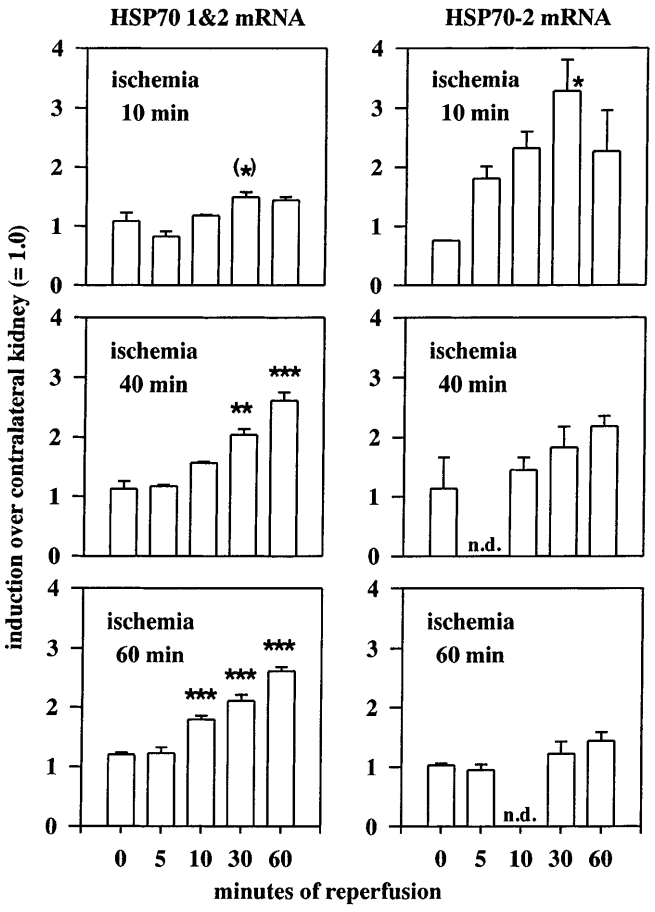

Fig. 1 Renal cortical mRNA expression of HSP70-1&2 "sum" and HSP70-2 "only" after 10, 40 or 60 min of warm renal ischemia and subsequent reperfusion. Values (5–10 per group) are normalized to GAP-DH mRNA and are presented relative to the respective mRNA ratio of HSP/GAP-DH in the contralateral nonischemic kidney. Significance of differences compared with the first postischemic value (=0 min of reperfusion) by ANOVA and subsequent individual comparisons: $*P = 0.06$, $*P > 0.05$, $**P < 0.011$, $***P < 0.001$; *n.d.* not determined

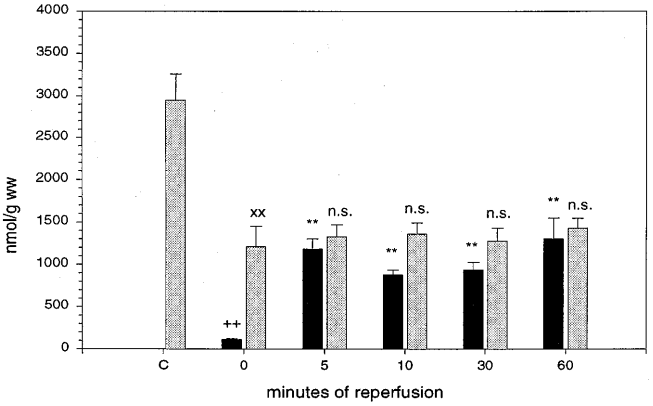

Fig. 2 ATP content in rat kidney after 10 min of warm ischemia and subsequent reperfusion (controls versus ischemic kidney: $^{++}P < 0.001$; ischemic versus reperfused kidney: $**P < 0.001$; reperfused kidney versus controls: $^{xx}P < 0.001$, *n.s.* nonsignificant). *C* controls nephrectomized immediately after anesthesia; *black columns* ischemic-reperfused kidney, *grey columns* nonischemic right kidney exposed to anesthesia for *x* min

compared with controls, but then HSP70-2 mRNA was expressed in a different pattern: During subsequent reperfusion after 10 min of ischemia HSP70-2 mRNA markedly increased until it peaked with a 3-fold higher level after 30 min of reperfusion (Fig. 1). After prolonged ischemia (40–60 min), the small changes in HSP70-2 mRNA values did not reach of significance (Fig. 1).

ATP

Compared with renal ATP levels in rat kidneys nephrectomized immediately after the induction of anesthesia, ATP levels in the untouched contralateral kidneys were substantially reduced throughout the experiment (Figs. 2–4), presumably due to the prolonged effects of anesthesia. Therefore, recovery of ATP levels in ischemic-reperfused kidneys is considered relative to these contralateral values.

After 10 min of ischemia, the renal ATP content was at approximately 5–10% of its original value and did not decrease any further with a longer period of ischemia. ATP recovery during subsequent reperfusion differed depending on the duration of the prior ischemic period as follows: After 10 min of ischemia, in the tenth minute of subsequent reperfusion there was already a recovery to 60% of the ATP content in the contralateral nonischemic control kidney that increased further to an almost full recovery by 60 min of reperfusion (Fig. 2). When the kidney was exposed to 40 or 60 min of ischemia ATP levels were regenerated up to only 50% of the ATP content of the contralateral nonischemic control kidney during reperfusion in our experiment, even though this value was reached already after 5 min of reperfusion (Figs. 3, 4).

Discussion

Only young rats were investigated, because the extent of stress-inducible HSP70 expression decreases continuously with age [20]. Since constitutive expression of HSP70 is usually at very low levels [11, 24], and some authors have even reported HSP70 to be undetectable in normal rat kidneys [12] or at least in the renal cortex [29], the amplification of mRNA by RT-PCR was the method of choice, and changes in HSP70 protein are paralleled in general by changes in HSP70 mRNA levels [26]. Homogenates of renal pole tissue (=mostly cortex) were investigated, since after ischemic injury differences in glomerular, tubular and interstitial expression are reported [5, 10, 16, 29]. Amounts of constitutively expressed HSP70 are lowest in the cortex compared with the inner medulla, where the highest values are found;

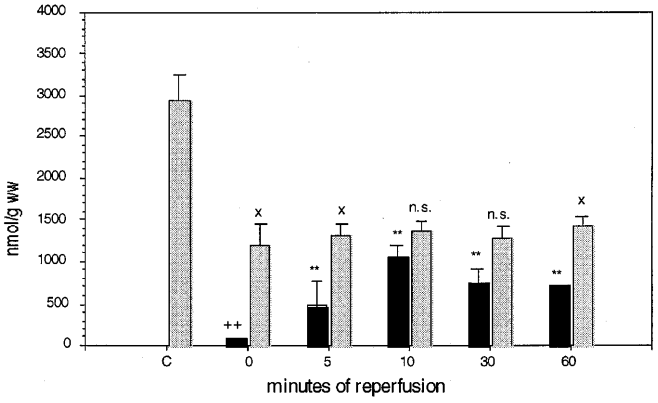

Fig. 3 ATP content in rat kidney after 40 min of warm ischemia and subsequent reperfusion (controls versus ischemic kidney: $^{++}P < 0.001$; ischemic versus reperfused kidney: $^{**}P < 0.001$; reperfused kidney versus controls: $^{x}P < 0.001$; *n.s.* nonsignificant). *C* controls nephrectomized immediately after anesthesia; *black columns* ischemic-reperfused kidney, *grey cloumns* nonischemic right kidney exposed to anesthesia for *x* min

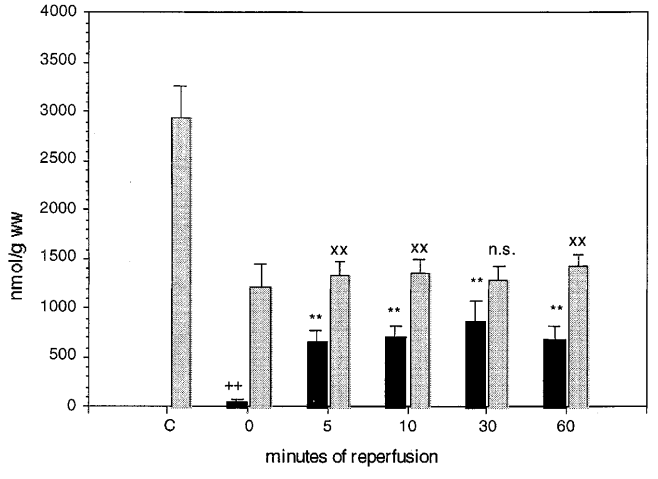

Fig. 4 ATP content in rat kidney after 60 min of warm ischemia and subsequent reperfusion (controls versus ischemic kidney: $^{++}P < 0.001$; ischemic versus reperfused kidney: $^{**}P < 0.001$; reperfused kidney versus controls; $^{xx}P < 0.001$; *n.s.* nonsignificant). *C* controls nephrectomized immediately after anesthesia; *black columns* ischemic-reperfused kidney, *grey columns* nonischemic right kidney exposed to anesthesia for *x* min

after ischemia even a decrease in HSP70 is observed in the inner medulla and during reperfusion original levels are barely reached [26]. As shown by Dodd et al. [10] by immunohistochemical staining, after ischemia-reperfusion injury cells other than the interstitial cells of the cortex are capable of expressing HSP70. Also, in normal human kidney, HSP72/73 showed uniform fine granular staining of visceral glomerular epithelial cells and epithelia of distal convoluted tubules and collecting ducts without localization in the proximal tubules [32]. Contralateral nonischemic kidneys served as controls for the HSP70 mRNA expression. This approach excludes potential confounding influences of drugs, anesthesia, laparatomy, temperature [6] and circadian rhythm [27] on the heat shock response in our experiments, which were performed at 0900–1100 hours.

Heat shock responses in rat kidney following ischemia-reperfusion have been analyzed mainly histochemically, there are no reports on the mRNA regulation of the two HSP70 genes. Only HSP70 mRNA values without identification of the gene source have been reported [2, 14]. No induction of HSP70 mRNA after 30 min of renal ischemia was detected by Northern blotting [2], but there was a strong induction during reperfusion, with a 12-fold increase after 1 h of reperfusion and a decline thereafter [2]. Gaudio et al. [14] described a 2-fold increase in HSP72 in immature rat tubulus cells after 45 min of anoxia and 20 min of reoxygenation. Similarly, in our experiments at the end of ischemic periods of different durations we could not find any significant changes in the abundance of HSP70/1&2 "sum" mRNA compared with nonischemic controls. Maintenance of the mRNA levels argues against a block of mRNA synthesis during ischemia. The fact that we found no induction immediately after ischemia agrees with previous investigations [2]. However, after 40 and 60 min of ischemia, HSP70/1&2 "sum" mRNA continuously increased with prolongation of reperfusion up to 1 h (Fig. 1). Bardella and Comolli [2] showed that at this time point of reperfusion the mRNA response is maximal, while other groups reported maximal induction of HSP protein later [12, 29]. In our young rats, maximal increase in HSP mRNA after postischemic reperfusion were lower than previously reported [2], presumably since young rats have higher levels of HSP and a very high antioxidative capacity [1] which attenuates the oxidative stress due to ischemia-reperfusion. After 10 min of ischemia, however, the increase in HSP70/1&2 "sum" mRNA during reperfusion was transient and barely detectable (Fig. 1). Emami et al. [12] could not detect any HSP70 protein induction after ischemia of less than 15 min.

Although there are to our knowledge no in vivo reports on HSP70 expression in humans after renal ischemia-reperfusion, HSP70 is known to be increased in patients with renal disease [10], after ischemia, toxin exposure and oxidative stress [19] and in human proximal tubular cell cultures after hypoxia-reperfusion at the mRNA level [30].

Regarding the analysis of the HSP70-2 "only" mRNA expression after brief ischemia, we propose an explanation for the existence of two different genes encoding the same protein. First, as shown in Fig. 1 after 10 min of ischemia, there is rarely a visible induction of HSP70-1&2 "sum" mRNA during reperfusion, in contrast to the remarkable increase in HSP70-2 mRNA. On the other hand, after longer periods of ischemia resulting in high amounts of mRNA for both genes, there is no significant increase in HSP70-2 mRNA during reperfusion. This means that even after a 3-fold induction, HSP70-2 is generally expressed at a much lower level than HSP70-1 (we also needed more ampli-

fication cycles), at least under the stress caused by ischemia-reperfusion – otherwise it would strongly influence the mRNA "sum" pattern of the two genes. This is different under heat shock: both the HSP70-1 and HSP70-2 genes are expressed at high levels in cells heat-shocked at 42°C [24]. Since both genes code for identical proteins with identical functions and capabilities, our results suggest that differential expression of the two genes could enable the cell to respond rapidly but precisely to different demands. Our data show that the *HSP70-2* gene is far more sensitive with a lower activation threshold and only works in a range of mild injury, while the *HSP70-1* gene mediates the major response after severe injury.

Our data also reveal that ATP levels in these tissues are in accordance with the HSP mRNA measured in our experiment. After brief ischemia (10 min), the ATP is almost fully recovered after 10 min of reperfusion in accordance with the downregulated HSP mRNA values shown in our experiment already after 30 min of reperfusion, while after longer ischemia ATP regeneration is only partial and after 60 min of reoxygenation the HSP70-1&2 mRNA is still increasing.

Since the ATP level drops rapidly after only brief ischemia and protein damage might require longer ischemia, we postulate that after brief ischemia the HSP70 mRNA is modulated by ATP depletion and that after longer ischemia protein aggregates are the more decisive trigger. Both conditions – ATP depletion [21] and accumulation of aggregates of denaturated proteins [22, 23] – are known to trigger heat shock factor activation [15], which is a prerequisite for induction of HSP expression.

It is known that during ischemia and in the early phase of reperfusion protein synthesis is downregulated. DeGracia et al. [7] showed that this is not due to an altered ribosomal function but that, on the contrary, ribosomes are fully functional, at least in vitro, during the whole reperfusion period. It was suggested that cessation of de novo protein synthesis is a protective mechanism to prevent misfolding of proteins and, nevertheless, a possible chaperone release of ribosome-associated HSP could support the cellular defense [34]. This also allows a plausible explanation for our observation that HSP70-1&2 mRNA levels remain constant during the period of ischemia, when protein synthesis is depressed, and that expression of these mRNAs is induced during reperfusion, when protein synthesis is reinitiated and the newly synthesized mRNAs can immediately be translated into HSP to protect the cells from reperfusion injury.

It was recently discovered that HSP70-2 is closely related to cell proliferation and apoptosis in that its absence or impaired function results in programmed cell death in various tissues [8, 9, 25]. This leads to an even more fascinating possible function in our experimental constellation: HSP70-2 induction after short stress (10 min ischemia and subsequent reperfusion) could render cells resistant to initiation of apoptosis, while after longer periods of ischemia large amounts of HSP chaperone (HSP70-1 mediated) are required for repair processes of surviving cells.

In conclusion, our data indicate that HSP70-1 and HSP70-2 proteins have differential roles during postischemic reperfusion in the renal cortex. HSP70-2 mediates a short and sensitive transient response, while HSP70-1 represents the major and long-lasting protective defense mechanism.

References

1. Akçetin Z, Busch A, Kessler G, Heynemann H, Holtz J, Brömme HJ (1999) Evidence for only moderate lipid peroxidation during ischemia-reperfusion of rat kidney due to its high antioxidative capacity. Urol Res 27:280–284
2. Bardella L, Comolli R (1994) Differential expression of c-jun, c-fos and hsp 70 mRNAs after folic acid and ischemia-reperfusion injury: effect of antioxidant treatment. Exp Nephrol 2:158
3. Beckmann RP, Lovett M, Welch WJ (1992) Examining the function and regulation of hsp-70 in cells subjected to metabolic stress. J Cell Biol 117:1137
4. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294
5. Cowley BD, Gudapaty S (1995) Temporal alterations in regional gene expression after nephrotoxic renal injury. J Lab Clin Med 125:187
6. Cullen KE, Sarge KD (1997) Characterization of hypothermia-induced cellular stress response in mouse tissues. Biol Chem 272:1742
7. DeGracia DJ, Grossmann LJ (1993) Studies of the protein synthesis system in the brain cortex during global ischemia and reperfusion. Resuscitation 25:161
8. Dix DJ, Allen JW, Collins BW, Mori C, Nakamura N, Poorman-Allen P, Goulding EH, Eddy EM (1996) Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc Natl Acad Sci USA 93:3264
9. Dix DJ, Allen JW, Collins BW, Poorman-Allen P, Mori C, Blizard DR, Brown PR, Goulding EH, Strong BD, Eddy EM (1997) HSP70-2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes. Development 124:4595
10. Dodd SM, Martin JE, Swash M, Mather K (1993) Expression of heat shock protein epitopes in renal disease. Clin Nephrol 39:239
11. Drummond IAS, Steinhardt RA (1987) The role of oxidative stress in the induction of *Drosophila* heat shock proteins. Exp Cell Res 173:439
12. Emami A, Schwartz JH, Borkan SC (1991) Transient ischemia or heat stress induces a cytoprotectant protein in rat kidney. Am J Physiol 260:F479
13. Hunt C, Morimoto RI (1985) Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc Natl Acad Sci USA 82:6455
14. Gaudio KM, Thulin G, Mann A, Kashgarian M, Siegel NJ (1998) Role of heat stress response in the tolerance of immature renal tubules to anoxia. Am J Physiol 274:F1029
15. Imuta N, Ogawa S, Maeda Y, Kuwabara K, Hori O, Ueda H, Yanagihara T, Tohyama M (1998) Induction of 72-kDa inducible heat shock protein (HSP72) in cultured rat astrocytes after energy depletion. J Neurochem 70:550
16. Komatsuda A, Wakui H, Imai H, Nakamoto Y, Miura AB, Itoh H, Tashima Y (1992) Renal localization of the constitutive 73-kDa heat-shock protein in normal and PAN rats. Kidney Int 41:1204
17. Leung TKC, Rajendran MY, Monfries C, Hall C, Lim L (1990) The human heat-shock protein family: expression of a novel

heat-inducible HSP70 (HSP70B′) and isolation of its cDNA and genomic DNA. Biochem J 267:125
18. Lisowska K, Krawczyk Z, Widlak W, Wolniczek P, Wisniewski J (1994) Cloning, nucleotide sequence and expression of rat heat inducible hsp-70 gene. Biochim Biophys Acta 1219:64
19. Lovis C, Mach F, Donati YR, Bonventre JV, Polla BS (1994) Heat shock proteins and the kidney. Ren Fail 16:179
20. Maiello M, Boeri D, Sampietro L, Pronzato MA, Odetti P, Marinari UM (1998) Basal synthesis of heat shock protein 70 increases with age in rat kidneys. Gerontology 44:15
21. Mestril R, Chi S-H, Sayen MR, Dillmann WH (1994) Isolation of a novel inducible rat heat shock protein (hsp70) gene and its expression during ischaemia/hypoxia and heat shock. Biochem J 298:561
22. Mifflin LC, Cohen RE (1994) Characterization of denatured protein inducers of the heat shock (stress) response in *Xenopus laevis* oocytes. J Biol Chem 269:15710
23. Mifflin LC, Cohen RE (1994) hsc70 moderates the heat shock (stress) response in *Xenopus laevis* oocytes and binds to denatured protein inducers. J Biol Chem 269:15718
24. Milner CM, Campbell RD (1990) Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics 32:242
25. Mori C, Nakamura N, Dix DJ, Fujioka M, Nakagawa S, Shiota K, Eddy EM (1997) Morphological analysis of germ cell apoptosis during postnatal testis development in normal and Hsp70-2 knockout mice. Dev Dyn 208:125
26. Muller E, Neuhofer W, Burger-Kentischer A, Ohno A, Thurau K, Beck F (1998) Effects of long-term changes in medullary osmolality on heat shock proteins HSP25, HSP60, HSP72 and HSP73 in the rat kidney. Pflugers Arch 435:705
27. Rensing L, et al (1996) Heat shock proteins and circadian rhythms. Chronobiol Int 13:239
28. Riabowol KT, Mizzen LA, Welch WJ (1988) Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. Science 242:433
29. Schober A, Muller E, Thurau K, Beck FX (1997) The response of heat shock proteins 25 and 72 to ischaemia in different kidney zones. Pflugers Arch 434:292
30. Turman MA, Kahn DA, Rosenfeld SL, Apple CA, Bates CM (1997) Characterization of human proximal tubular cells after hypoxic preconditioning: constitutive and hypoxia-induced expression of heat shock proteins HSP70 (A, B, C), HSC70 and HSP90. Biochem Mol Med 60:49
31. Venkatachalam MA, Patel YJ, Kreisberg JI, Weinberg JM (1988) Energy thresholds that determine membrane integrity and injury in a renal epithelial cell line (LLC-PK1): relationships to phospholipid degradation and unesterified fatty acid accumulation. J Clin Invest 81:745
32. Venkataseshan VS, Marquet E (1996) Heat shock protein 72/73 in normal and diseases kidneys. Nephron 73:442
33. Walter L, Rauh F, Günther E (1994) Comparative analysis of the three major histocompatibility complex-linked heat shock protein 70 genes of the rat. Immunogenetics 40:325
34. Westwood JT, Clos J, Wu C (1991) Stress induced oligomerization and chromosomal relocalisation of heat shock factor. Nature 353:822