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Induction of heat-shock proteins does not prevent renal tubular injury following ischemia

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Induction of heat-shock proteins does not prevent renal tubular injury following ischemia. The possible protective effect of heat-shock proteins (HSPs) on ischemic injury to renal cells was assessed in two different experimental models: ischemia-reflow in intact rats and medullary hypoxic injury as seen in the isolated perfused rat kidney. Heat shock was induced by raising the core temperature of rats to 42°C for 15 minutes. Following this, Northern blots showed enhanced gene expression of HSP70, HSP60 and ubiquitin at one hour and reaching a maximum by six hours after heat shock in all regions of the kidney, but most prominently in medulla and papilla. The HSP70 protein in the kidney, estimated by immunohistochemical means, was detectable 24 hours following heat shock and further increased at 48 hours following heat shock. In the first set of experiments, the animals underwent uninephrectomy followed by cross clamping of the remaining renal artery for 40 minutes prior to reflow. Serum creatinine and urea nitrogen rose to 3.15 ± 0.98 and 126.4 ± 62.5 mg/dl at 24 hours. No significant differences were observed at 24, 48 and 72 hours after reflow between these values in control rats and rats pretreated with heat shock 48 hours earlier. Severe morphological damage to proximal tubules of the renal cortex was observed to the same extent in both groups. In a second set of experiments, the right kidney was removed either 24 or 48 hours after heat shock and perfused in isolation for 90 minutes. Functional and morphological parameters were compared with those of isolated perfused kidneys obtained from animals that had not been subjected to heat shock. No difference was observed in the degree or extent of hypoxic injury to the medullary thick ascending limb, characteristically observed in the isolated perfused rat kidney, nor did prior induction of HSPs modify the progressive decline in glomerular filtration rate or fractional reabsorption of glucose seen in perfused kidneys. Fractional reabsorption of sodium was slightly higher in kidneys from rats earlier exposed to heat shock. These results do not support the hypothesis that heat shock proteins prevent ischemic renal injury.

Rapid recovery following ischemic renal failure requires preservation of the integrity of renal epithelial cells. A major role in protection against injury in all living cells is thought to be played by the heat shock proteins (HSPs), the synthesis of which is increased after cells are exposed to high temperatures (42°C) for a restricted time. In mammalian cells, HSPs are commonly classified according to their size, for example, HSP 20 to 30 kDa, HSP 60, HSP 70, HSP 90 [1, 2]. Although many of their functions are still speculative, some properties have been clearly defined. HSPs are involved in protein folding and unfolding, thus providing protection against denaturation [1, 3]. They may also play a role in the transport of newly synthesized polypeptides into mitochondria and endoplasmic reticulum [2]. Other groups of HSPs, especially ubiquitin, are thought to participate in the degradation of denatured proteins [4]. The induction of HSPs by heat causes cells to become thermotolerant; that is, able to survive higher temperatures than can untreated cells [2, 5]. Resistance to other stressful conditions, including exposure to heavy metals [6] and hypoxia [7] may be enhanced by HSPs.

Although clear evidence that HSPs protect against non-thermal injury has been mainly restricted to cultured cells, it has been suggested that they may play a similar role in preventing ischemic damage to intact organs, including brain [8], heart [9], and kidney [10].

The present experiments were performed to determine whether induction of HSPs might modify ischemic injury to the kidney. Two models of renal damage were examined: ischemia-reflow in intact rats and hypoxic injury to the medullary thick ascending limb (mTAL) as seen in the isolated perfused rat kidney [11].

Methods

Animals

Male Sprague-Dawley rats, with an average weight of 340 g (range 300 to 400 g) fed on Purina Lab Chow *ad libitum* were used for all experiments.

Heat-shock protein induction

Animals were anesthetized with Brevital® (dose 1 mg/kg) and placed in a water bath maintained at a constant temperature of 42°C. They were kept in the water bath until core temperature (measured by deep rectal thermometer) had reached 42 ± 0.5°C, remaining there for 15 minutes; this usually took 45 to 55 minutes. The animals received 1.5 to 2 ml 0.9% saline intraperitoneally immediately before and after heat exposure. They were then dried and placed under an infrared lamp until they awoke. Control animals were similarly anesthetized but maintained in a warm chamber at 37°C rather than 42°C. The animals were then utilized either for experiments examining *in vivo* renal ischemia or isolated

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renal perfusion experiments carried out 24 or 48 hours following heat shock (see below).

Determination of heat-shock protein mRNA induction

Animals were sacrificed either before (Co), immediately after (0), or 1, 3, 6, 24 and 48 hours after heat shock. Kidneys were removed immediately, dissected on ice into cortex, outer medulla and inner medulla (papilla) and snap frozen. Both kidneys from two animals were pooled in order to obtain sufficient tissue for the preparation of RNA from medulla and papilla.

RNA preparation and Northern blot analysis

RNA was prepared as previously described [12]. Briefly, snap-frozen tissue was homogenized in GIT buffer (4 M guanidine isothiocyanate/0.5% sodium N-lauryl sarcosine/25 mM sodium citrate/0.1 M β-mercaptoethanol, pH 7.0) using a homogenizer from Brinkman Instruments. RNA was then purified on cesium chloride gradients. Ten micrograms of total RNA from papilla, medulla and cortex were electrophoresed on a 1% agarose/formaldehyde gel, transferred to a nylon membrane (Gene Screen, NEN Research Products, Boston, MA, USA) and successively hybridized with cDNA probes for both inducible and constitutive HSP 70 (pHS709, pHS710 [13]), HSP 60 (pHS601 [13]), ubiquitin [14] and GAPDH [15] labeled by random priming. mRNA levels were quantified using the ImageQuant software and standardized to the level of GAPDH.

Determination of HSP 70 protein induction

HSP 70 protein expression was determined by immunohistochemistry 24 and 48 hours after heat shock.

Kidneys were perfusion-fixed with phosphate buffered formalin, kept in buffered formalin solution for one hour, and then transferred to phosphate buffer at 4°C for approximately 48 hours. Cross sectional slices of the kidney were then submitted for routine histologic processing and paraffin embedding. Sections were cut at a thickness of 4 μ, mounted on silanated slides, air dried, and then baked for 10 minutes at 57°C. Antigen retrieval was performed on sections on slides by microwaving in a citric acid buffer twice for five minutes. Slides were then immunostained by the peroxidase-antiperoxidase method, using a mouse monoclonal antibody, H7 F4–2 (obtained from Dr. W. Welch, U. of California, San Francisco, CA, USA), directed against both constitutive and inducible members of the HSP 70 family (HSP 73 and HSP 72). The HSP 70 specificity of this antibody in paraffin sections has been previously demonstrated [16]. A primary antibody dilution of 1:300 was used, followed by rabbit anti-mouse (Dako) at 1:50 × 1 hour, then peroxidase-antiperoxidase complex (mouse clono-Pap, Sternberger-Meyers) at 1:200 × one hour, and finally by development in 50 mg/ml diaminodenzidine for approximately nine minutes and counterstaining in 10% hematoxylin. Staining was compared only among slides stained in the same run. Photomicrography was performed at 10× with Kodak TMX-100 professional black and white film using a #47 Wratten filter (Kodak catalogue #149 5787) to maximize the contrast of immunostaining relative to counterstaining.

Ischemia experiments

Animals were anesthetized with Brevital® (dose 1 mg/kg). Through an abdominal midline incision, the right kidney was removed. The left renal artery was then clamped for 40 minutes. After release of the clamp, the abdominal incision was closed and the animals were allowed to awaken. Samples of blood for determination of plasma urea nitrogen and creatinine were taken before clamping the left renal artery, and 24, 48 and 72 hours after ischemia. Volume losses were replaced by intraperitoneal saline. After 24 or 72 hours the rats were again anesthetized and the left kidney was perfusion-fixed for histological evaluation. This was completed by Dr. S. Rosen in a blinded fashion. The extent of severe damage of the proximal tubules was graded in the following manner: 25 points if 0 to 25% of the counted tubules were involved by severe changes (blatant mitochondrial swelling with extensive nuclear pyknosis and cell fragmentation), 50 points for 25 to 50%, 75 points for 50 to 75%, 100 points for 75 to 100% and 125 points if the medullary rays were also involved. Approximately 120 tubules (124 ± 21 mean ± SD) were evaluated per kidney. In order to exclude damage to the kidney by heat exposure itself, four kidneys were perfusion-fixed 48 hours after heat exposure and evaluated in the same way.

Isolated perfused kidney experiments

Isolated perfusion of the right kidney was performed according to the method of Ross, Epstein and Leaf [17]. Kidneys were perfused at 37°C with a Krebs-Ringer-Henseleit solution containing bovine serum albumin at a concentration of 67 g/liter and glucose at 5 mM, gassed with 5% CO_2/95% O_2 at a pH of 7.4. All perfusions were carried out for 90 minutes. After perfusion kidneys were perfusion-fixed for histologic evaluation.

The histological evaluation was completed by Dr. S. Rosen in a blinded fashion. Three zones of the inner stripe were analyzed: upper third (A) included all medullary thick ascending limbs (mTALs) intersecting a line immediately adjacent to the outer stripe (within 0.2 mm); middle third (B) was all mTALs intersecting a line drawn midway between the borders of the inner stripe; and lower third (C) was all mTALs intersecting a line immediately adjacent to the inner medulla (within 0.2 mm). These points were chosen for analysis because they provided areas in which topographical landmarks were easily ascertained. A percentage score was used to indicate the fraction of tubules involved with minimal to mild changes (chromatin margination, minor degrees of mitochondrial swelling), moderate changes (blatant mitochondrial swelling with extensive nuclear pyknosis) and severe changes (blatant mitochondrial swelling with extensive nuclear pyknosis and cell fragmentation). Approximately 140 tubules (142 ± 24 mean ± SD) were evaluated per kidney.

Statistical procedures

Statistical significance between untreated and heat shock treated animals was tested by multivariant ANOVA.

Results

Induction of heat-shock mRNA expression after exposure to heat

At baseline the messages for HSP 60 and both the inducible and constitutive forms of HSP 70 were barely detectable (Figs. 1–3, lane 1). However, following exposure of rats to heat all three HSPs were up-regulated, with maximal expression between one and six hours, followed by return to baseline at 24 hours (Figs. 1–3, lanes 3–5; Fig. 4). Interestingly, the increase in mRNA for all HSPs examined was substantially more prominent in medulla and papilla than in the cortex, with the greatest increases occurring in

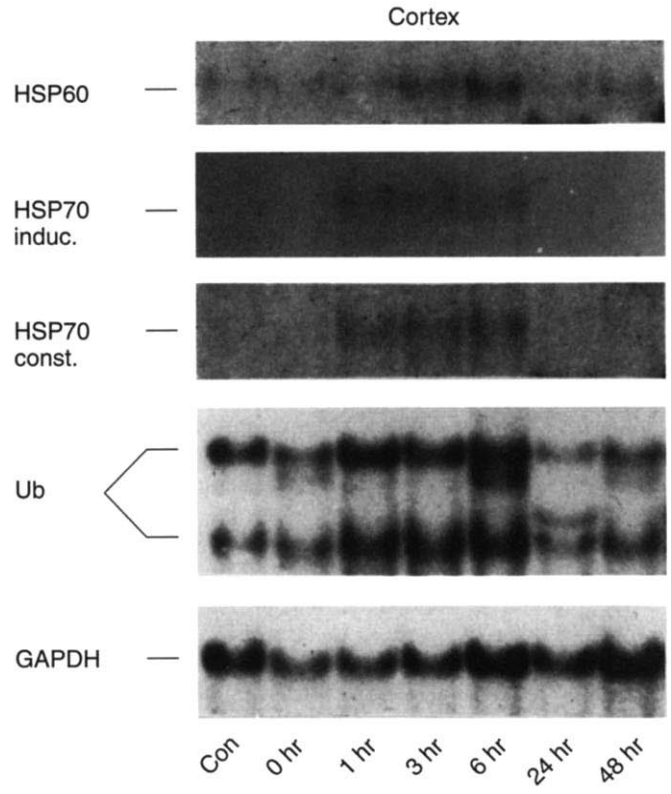

Fig. 1. *Induction of HSPs in renal cortex by heat shock.* Northern blots of renal outer cortex hybridized with cDNA for inducible and constitutive HSP 70, HSP 60, ubiquitin and GAPDH. Co are sham treated control animals; 0, 1, 3, 6, 24, 48 are animals sacrificed 0 hours, 1 hour, 3 hours, 6 hours, 24 hours and 48 hours following heat shock. Each lane represents the pooled total RNA from 3 animals.

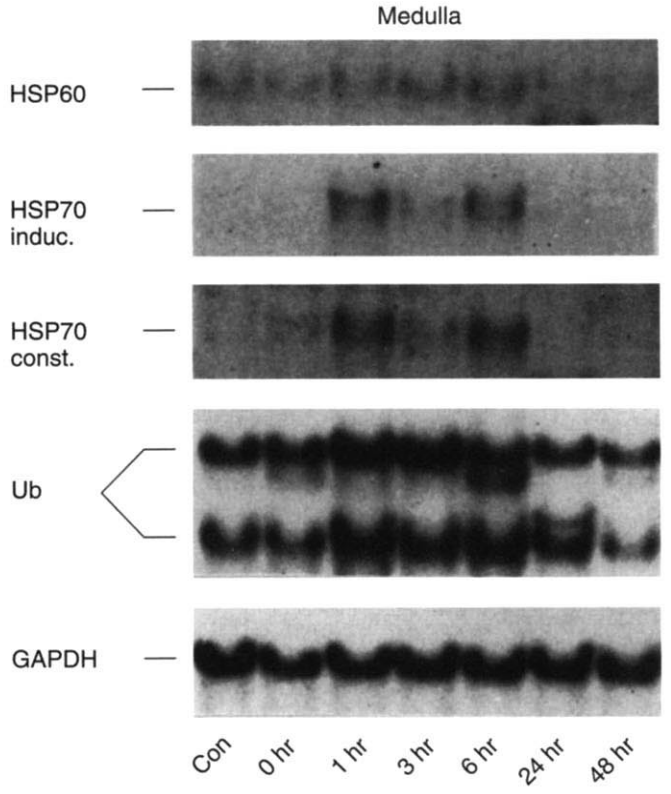

Fig. 2. *Induction of HSPs in renal medulla by heat shock.* Northern blots of renal outer medulla hybridized with cDNA for inducible and constitutive HSP 70, HSP 60, ubiquitin and GAPDH. Each lane represents the pooled total RNA from 3 animals.

the papilla (Fig. 4). The expression of ubiquitin was significantly greater at baseline than that of other HSPs tested, with an additional increase following heat shock.

Heat-shock protein expression after heat exposure

Immunohistochemistry showed consistently higher levels of HSP 70 immunostaining in kidneys 24 and 48 hours after heat exposure than in controls not exposed to heat (Fig. 5A). Control kidneys showed a moderate level of staining presumably due to constitutive HSP 73 expression. The increase in HSP70 immunostaining was much more pronounced at 48 hours than at 24 hours, with changes readily visible in inner medullary collecting ducts, medullary thick ascending limbs, and proximal tubules (Fig. 5 A, B). A modest increase in staining was also observed in glomeruli (Fig. 5A, first row). In the controls and 24 hour kidneys, immunostaining was localized primarily to the cytoplasm rather than to the nuclei, which were delineated by the hematoxlyn counterstain (Fig. 5B, first and second columns). At 48 hours, all tubules showed significantly more cytoplasmic as well as nuclear immunostaining. At this time, immunostaining was sufficiently intense to obscure the counterstain, and nuclei were therefore not as readily visualized as they were at 24 hours (Fig. 5B, third column).

Effect of prior heat stress on intact kidneys subjected to ischemia-reflow

In control animals not previously subjected to heat stress, serum creatinine rose from 0.44 ± 0.05 to 3.15 ± 0.98 mg/dl, and urea nitrogen from 17.3 ± 2.6 to 111.4 ± 24.6 mg/dl, 24 hours after the remaining left kidney had been clamped for 40 minutes. Serum urea and creatinine declined gradually during the following two days, as illustrated in Figures 6 and 7. In rats subjected to heat shock 48 hours before ischemia reflow the initial rise in serum creatinine was identical to that seen in the sham controls. The gradual decline in levels of urea and creatinine observed over the following two days did not differ significantly in rats pretreated with heat shock from controls.

Morphological evidence of severe renal injury was widespread in both groups of rats subjected to ischemia-reflow (Fig. 8). No difference in renal damage at 24 or 72 hours was apparent between the groups; tubular swelling, fragmentation and necrosis was as marked and extensive in the kidneys of rats that had been subjected to heat stress as in their controls. Kidneys subjected only to heat exposure without renal artery clamping showed no or only minimal tubular changes in the S3 segments.

Effect of prior heat stress on renal injury produced by isolated perfusion

As expected, in control kidneys subjected to isolated perfusion for 90 minutes C_{In} declined progressively together with a gradual fall in fractional reabsorption of sodium and a more pronounced decline in fractional reabsorption of glucose (Table 1). These functional changes are believed to reflect hypoxic damage seen most prominently in outer medullary tubules including the thick ascending limb and the straight portion of the proximal tubule (S3) [11]. Prior induction of heat-shock proteins did not alter the

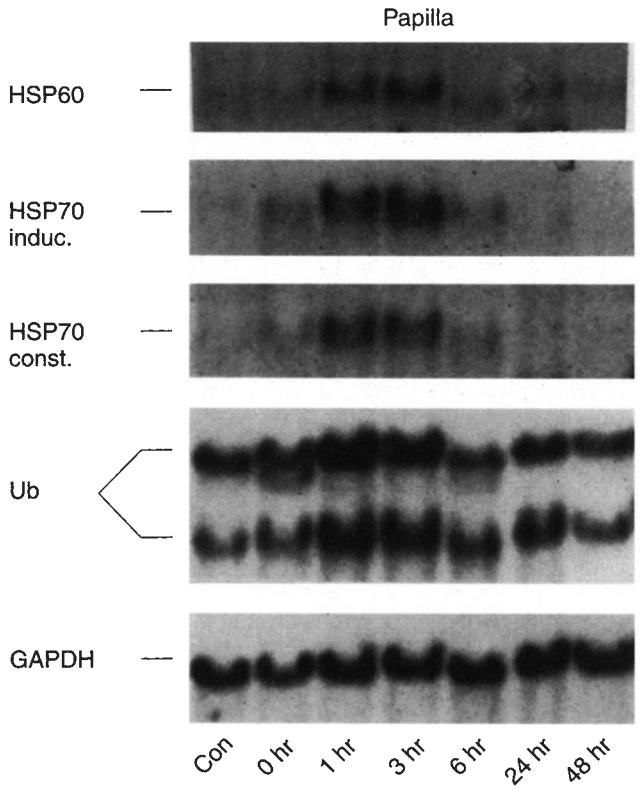

Fig. 3. *Induction of HSPs in renal papilla by heat shock.* Northern blots of renal papilla hybridized with cDNA for inducible and constitutive HSP 70, HSP 60, ubiquitin and GAPDH. Each lane represents the pooled total RNA from 3 animals.

progressive fall in C_{In} and fractional reabsorption of glucose found in controls, although fractional reabsorption of sodium was better maintained than in the perfused kidneys of rats that had not been exposed to heat. The degree and extent of morphological damage to cells lining the medullary thick ascending limb were similar in perfused kidneys with and without prior heat shock (Table 2).

Discussion

Cellular stress has been shown to induce rapid cellular synthesis of heat-shock proteins that are thought to facilitate folding and unfolding of proteins and their transport across the membranes of intracellular organelles and the endoplasmic reticulum [4]. Their protective effect has been most clearly established for HSP 70 by the findings that microinjection of antibodies to HSP 70 [18] or inhibition of its gene transcription cause thermosensitivity [19], whereas overexpression of HSP 70 in mammalian cells confers heat resistance [20, 21]. It has also been suggested that the cytoprotection provided by HSPs is non-specific, that is, they may improve cellular tolerance to a variety of repeated insults. This hypothesis is supported by the observation that thermotolerance and HSP expression can also be induced by other noxious stimuli such as ethanol or sodium arsenite [22]. Initial exposure to ischemia also stimulates formation of HSPs in kidney [10], brain [8, 23], liver [24] and heart [25]. In the heart and brain, where this has been the most extensively studied, it has been shown that prior exposure to ischemia confers enhanced resistance to subsequent ischemic events [26, 27]. The possibility that this improved resistance to ischemia is provided by HSP production is suggested by experiments in which pretreatment of animals with heat shock similarly protected against subsequent ischemic injury in the heart and brain [28–31]. Furthermore, in cultured cardiac myocytes enhanced production of HSP 70 increased resistance to anoxic injury [7].

In the kidney, initial exposureto toxins such as aminoglycosides, heavy metals or glycerol blunts the injury produced by rechallenge [32]. Exposure to one toxin is said to increase renal resistance to a second unrelated toxin, suggesting that the kidney has acquired a functional cytoprotectant [32]. That HSPs might provide this cytoprotection in the kidney was suggested by Emami, Schwartz and Borkan, who showed induction of HSP 70 in rat kidneys by both heat and ischemia [10].

In the present experiments, elevating core temperature to 42 ± 0.5°C induced increased gene expression of HSP 60 and ubiquitin, as well as of the inducible and constitutive forms of HSP 70. The pattern was quite uniform for all four probes used, characterized by an increase starting one hour and peaking between three and six hours after heat exposure. Twelve hours later mRNA returned to baseline levels or became undetectable. In agreement with previous reports for HSP 70 [10], induction of the message was more pronounced in papilla and outer medulla, and peaked earlier in papilla than in cortex. The expression of the HSP 70 proteins in renal cells showed a more prolonged time course with the greatest level of detectable protein at 48 hours after heat exposure (Fig. 5). Emami et al also noted prolonged expression of HSP 70, with detectable protein up to 10 days after heat stress [10].

Despite the clear-cut induction of heat-shock proteins by heat exposure in the intact rat, subsequent clamping of the renal artery produced a rise in plasma urea and creatinine that was similar to the changes seen in clamped controls, and a level of renal tubular injury at 24 hours after ischemia-reperfusion that was histologically indistinguishable from that in controls (Fig. 8). Similarly, when isolated kidneys from rats subjected to prior heat shock were perfused, they demonstrated the same progressive decline in GFR and tubular reabsorption of glucose, and the same degree of damage to medullary thick ascending limbs, as were seen in perfused control kidneys from rats not exposed to heat. The higher levels of fractional sodium reabsorption found in perfused kidneys from heat shocked rats may reflect their exposure *in vivo* to salt-retaining hormones stimulated by heat exposure. Thus, the induction of heat-shock proteins by prior exposure to heat did not enhance resistance to ischemic renal injury in cortex or medulla, tested in two different experimental models. In both models, anoxic injury can be ameliorated by a variety of other maneuvers well documented in the literature [33, 34], demonstrating that the severity of injury is not so pronounced as to prevent any and all protective influences.

Published experiments on the possible protective effects of heat shock in the kidney are scanty and conflicting. Chatson et al [35] reported that the rise in serum creatinine after 60 minutes of renal ischemia in rats was blunted by 8 to 11 minutes of prior heat exposure, but not by 12 to 15 minutes. Although Perdrizet et al [36] reported that the application of heat stress to pig kidneys prior to an ischemic insult would protect renal allografts from injury, their data in fact showed no statistically significant difference between kidneys from heat shocked animals and sham treated controls. Hyperthermia-induced expression of heat shock proteins was found by Zager et al [37] to exert only a trivial

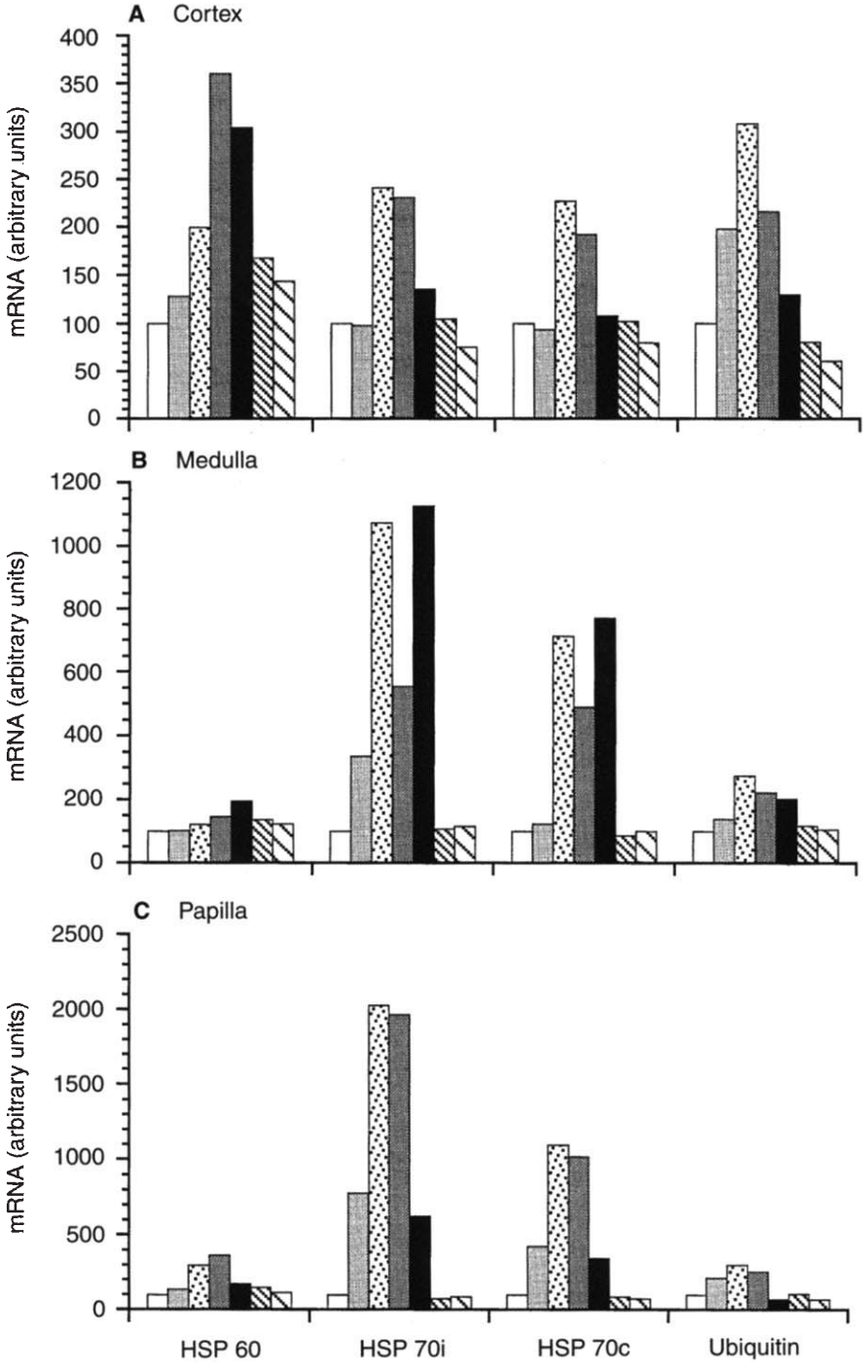

Fig. 4. *Quantification of mRNA for HSPs in the kidney.* Northern blots were analyzed using the ImageQuant software and the level of mRNA reported as arbitrary units/GAPDH for each message examined. Symbols are: (□) Control; (▨) 0 hr; (▩) 1 hr; (■) 3 hr; (■) 6 hr; (▧) 24 hr; (▨) 48 hr.

protective effect on the viability of proximal tubular segments exposed to hypoxia/reoxygenation *in vitro*.

The failure of heat-shock proteins to protect kidneys against ischemic stress, as demonstrated in the present experiments, might have several explanations. It is conceivable that we missed the time point of maximal protection by HSP induction, because this appears to vary widely depending on the animal species and the experimental model employed. For example, in isolated perfused rat hearts maximal protection was observed 48 hours after heat shock [9], while reduction of the size of myocardial infarcts in rabbits could only be observed 24 hours but not 40 hours after heat shock [25]. For this reason, we performed immunohistochemical staining to determine the time course of expression of HSP 70 protein in the kidney following heat shock. Because protein expression was greater at 48 hours than at 24 hours (Fig. 5), subsequent *in vivo* and *in vitro* ischemia experiments were performed at this time. However, no protection from injury was observed in the *in vivo* model of ischemic injury 48 hours after heat shock or the isolated perfused kidney model of anoxic injury at either 24 hours or 48 hours following heat shock.

A possible explanation for the lack of protection by HSPs might rest on the heterogeneity of the kidney as compared to the more

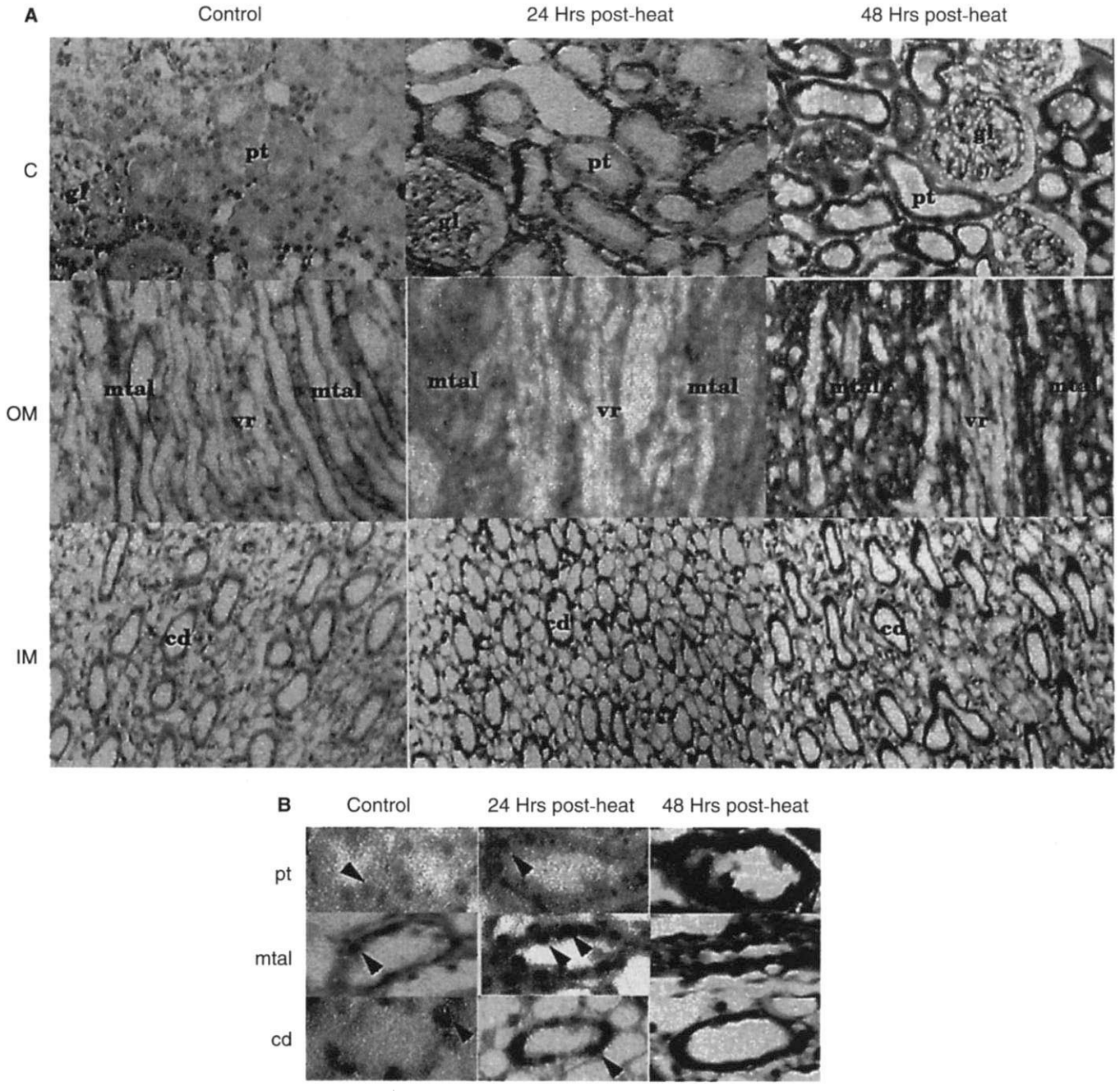

Fig. 5. A. *Immunohistochemical detection of HSP 70 induction in the kidney.* Immunohistochemical staining for HSP 72 and 73 in formalin fixed paraffin sections was performed. First column, controls; second column, 24 hours after heat exposure; third column, 48 hours after heat exposure. First row, C = cortex; gl = glomeruli; pt = proximal tubules. Second row, OM = outer medulla; mtal = medullary thick ascending limb; vr = vasa rectae. Third row, IM = inner medulla; cd = collecting ducts. Final magnification = 104×. **B.** Higher power view of HSP 70 immunostaining in representative tubules. Arrowheads show examples of counterstained cell nuclei delineated by the hematoxlyn counterstain. Columns as in A (p.h. = post heat). First row, proximal tubules (pt). Second row, medullary thick ascending limbs (mtal). Third row, inner medullary collecting ducts (cd). Final magnification = 182×.

homogeneous nature of the heart. In renal ischemia the majority of the tubular injury occurs in the S3 segment of the proximal tubule and the medullary thick ascending limb of Henle, with little injury in the superficial cortex. Therefore, it was important to determine if these regions of the kidney actually demonstrated up-regulation of the HSPs following heat treatment. Although our dissection technique excludes the inner cortex (containing the S3 segment) so as to avoid contamination by the outer medulla, the medullary expression of HSPs was found to increase 10-fold following heat shock (Fig. 4). This correlated well with the expression of the HSP 70 proteins which were most pronounced in the inner medullary collecting ducts and thick ascending limb (though apparent in other portions of the nephron including the proximal tubule, Fig. 5). Despite this documented up-regulation of the HSP 70 proteins in the mTAL and proximal tubule, no protection was observed in damage or functional parameters following ischemia.

Finally, the present results suggest that the stress proteins (and

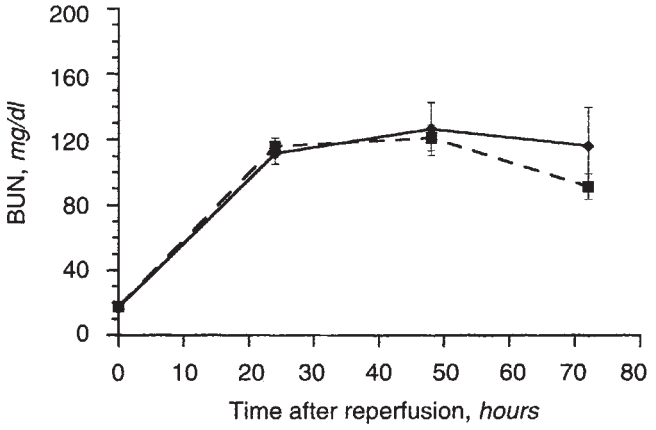

Fig. 6. *BUN values following ischemia/reflow in the rat.* BUN was determined 24, 48 and 72 hours after reperfusion following 40 min of renal ischemia. Values are means ± SEM. Symbols are: (-■-) animals pretreated with heat exposure 48 hours before ischemia/reperfusion ($N = 11$), (-◆-) sham treated animals ($N = 15$). P = NS between the two groups.

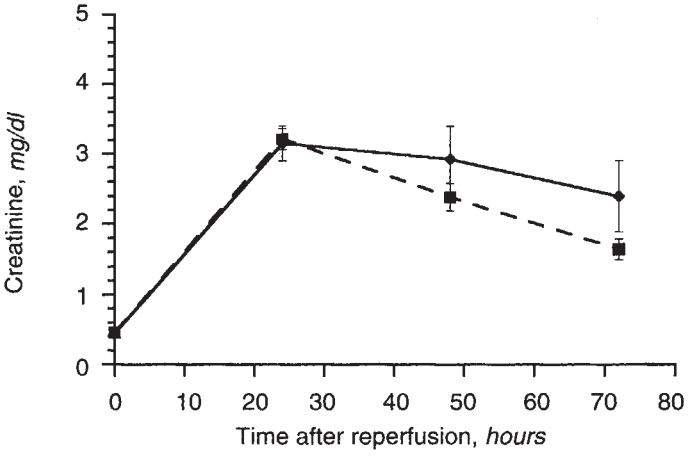

Fig. 7. *Creatinine values following ischemia/reflow in the rat.* Serum creatinine levels were determined in the same manner as BUN. Values are means ± SEM. Symbols are: (-■-) animals pretreated with heat exposure 48 hours before ischemia/reperfusion ($N = 11$), (-◆-) sham treated animals ($N = 15$). P = NS between the two groups.

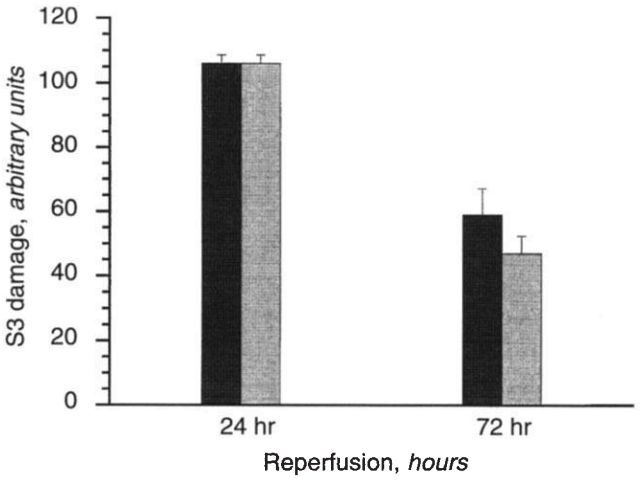

Fig. 8. *Histologic examination of the proximal tubule following ischemia/reflow.* Quantification of damage to the S3 segment of the proximal tubule in animals treated with ischemia (40 min)/reperfusion was performed. Damage is given in arbitrary units. Values are means ± SEM. Symbols are: (■) animals pretreated with heat exposure 48 hours before ischemia/reperfusion ($N = 4$), (▨) sham treated animals ($N = 4$).

Table 1. Functional parameters of the isolated perfused kidney during 90 min of perfusion

	Perfusion flow	C_{In}	Tr_{Na}	Tr_{Glu}
	ml/min		*%*	
30 min				
Co	26.8 ± 1.47	0.45 ± 0.02	92.5 ± 0.62	94.8 ± 1.18
HS 24h	28.8 ± 1.36	0.40 ± 0.03	94.1 ± 0.63	91.6 ± 2.43
HS 48h	30.1 ± 1.31	0.50 ± 0.03	94.8 ± 0.60[a]	96.1 ± 0.90
60 min				
Co	30.2 ± 1.44	0.27 ± 0.02	91.7 ± 1.43	77.7 ± 2.60
HS 24h	31.1 ± 1.16	0.21 ± 0.03	93.5 ± 0.92	73.7 ± 3.65
HS 48h	32.6 ± 1.23	0.29 ± 0.02	94.4 ± 0.60[a]	80.2 ± 2.28
90 min				
Co	32.4 ± 1.41	0.19 ± 0.01	90.7 ± 1.63	67.9 ± 2.16
HS 24h	33.4 ± 1.06	0.17 ± 0.03	93.1 ± 1.25	63.3 ± 3.81
HS 48h	34.4 ± 1.57	0.21 ± 0.02	94.1 ± 0.74[a]	67.0 ± 3.39

Data are means ± SEM.

Abbreviations are: Co, sham operated animals ($N = 8$); HS 24h, animals pretreated with heat exposure 24 hours before perfusion ($N = 5$); HS 48h, animals pretreated with heat exposure 48 hours before perfusion ($N = 8$).

[a] $P < 0.05$ compared to controls

perhaps their distribution) [38], induced in the kidney by hypoxia on the one hand and by heat stress on the other, are not identical. Several studies have demonstrated that sub-lethal exposure of kidneys to a noxious stimulus induces tolerance to a subsequent similar exposure. Heat shock protects against heat [4, 21] and ischemic stress partially protects against subsequent ischemia [39]. Nevertheless, cross-tolerance cannot be assumed as a general principle. In endothelial cells, for example, hypoxia has been shown to induce the expression of proteins different from those associated with heat shock. Inability of cells to express these "hypoxia-associated proteins" greatly reduces their resistance to hypoxic stress [40].

The present experiments do not exclude the possibility that the heat-shock proteins may play a limited role in moderating less severe renal tubular insults in which cells are not fated for death, or that they might help to accelerate repair following ischemic injury. However, if such an effect was present its magnitude was small. The eventual utility of induction of HSPs as a means to prevent ischemic renal injury appears limited by the lack of ability of HSPs to prevent actual cell death and the need for induction of HSPs prior to the injury.

Acknowledgments

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Reprint requests to Franklin H. Epstein, M.D., Beth Israel Hospital, DA517, 330 Brookline Avenue, Boston, Massachusetts, USA.

Table 2. Histologic evaluation of isolated perfused kidneys after 90 minutes of perfusion

	Zone A	Zone B	Zone C
Severe			
Co	4.2 ± 2.3	78.1 ± 5.2	70.3 ± 9.6
HS 24h	10.8 ± 4.8	82.8 ± 3.9	80.0 ± 4.0
HS 48h	10.5 ± 5.8	88.0 ± 3.2	76.4 ± 11.4
Moderate			
Co	0.1 ± 1	0 ± 0	5.2 ± 3.8
HS 24h	0 ± 0	0 ± 0	0 ± 0
HS 48h	0 ± 0	0.5 ± 0.5	3.0 ± 2.2
Mild			
Co	95.7 ± 2.4	21.9 ± 5.2	24.6 ± 6.9
HS 24h	89.2 ± 4.8	17.2 ± 3.9	20.1 ± 4.0
HS 48h	89.5 ± 5.7	11.6 ± 3.1	20.6 ± 10.0

Zones A, B, C refer to the upper third, middle third and lower third of the inner stripe.

Data are mean percentage of tubules demonstrating severe, moderate or mild injury ± SEM.

Abbreviations are: Co, sham treated animals ($N = 8$); HS 24h, animals pretreated with heat exposure 24 hours before perfusion ($N = 5$); HS 48h, animals pretreated with heat exposure 48 hours before perfusion ($N = 8$).

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