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# Ischemic Conditioning Prevents Na,K-ATPase Dissociation from the Cytoskeletal Cellular Fraction after Repeat Renal Ischemia in Rats

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## ABSTRACT

Recent studies have suggested that heat shock proteins (HSPs) are involved in the restoration of the cytoskeletal anchorage of Na,K-ATPase after renal ischemia. To determine their role in ischemic conditioning, we investigated whether cytoskeletal Na,K-ATPase was stabilized during repeat ischemia concurrent with 25-kD and 70-kD HSPs induction. Anesthetized rats either underwent single unilateral renal ischemia or were conditioned with bilateral renal ischemia and, after 18 h of reflow, were then subjected to repeat unilateral renal ischemia. Renal cortex was harvested, and effects of single *versus* repeat ischemia were compared by Triton X-100 extraction, by immunohistochemistry, and by an *in vitro* assay of Na,K-ATPase association with isolated cytoskeletal fractions. In contrast to single ischemia, repeat ischemia did not result in increased Triton X-100 extractability of Na,K-ATPase. Levels of 25-kD and 70-kD HSPs were significantly induced by ischemic conditioning and redistributed into the cytoskeletal fraction after single and repeat ischemia. Immunohistochemistry also showed significant disruption of Na,K-ATPase within proximal tubules only after a single episode of ischemia, whereas repeat ischemia did not alter the pattern of restored Na,K-ATPase localization in conditioned renal cortex. The preserved association of Na,K-ATPase with the cytoskeletal fraction of conditioned renal cortex was effectively abolished *in vitro* by addition of antibodies against 25-kD or 70-kD HSP. These results suggest that 25-kD and 70-kD HSPs induced by ischemic conditioning stabilize the cytoskeletal anchorage of Na,K-ATPase during repeat renal ischemia. **(*Pediatr Res* 51: 722–727, 2002)**

**Abbreviations**

**HSP,** heat-shock protein
**ECL,** enhanced chemiluminescence

Renal ischemia not only disrupts cellular homeostasis and causes injury but also induces a cellular stress response that participates in repair processes and may protect specific cellular structures against subsequent injury (1). Renal ischemia results in a transient relocation of Na,K-ATPase into the apical membrane domain in the proximal tubule cell (2, 3). This dislocated Na,K-ATPase remains functional but uses ATP to pump sodium back into the tubule lumen (2). Several clinical sequelae such as renal sodium loss and reduced glomerular filtration as a result of tubuloglomerular feedback can be explained by this redistribution of Na,K-ATPase.

For Na,K-ATPase to be translocated, it must first dissociate from its cytoskeletal anchorage (2–5). Triton X-100 extraction fractionates the cellular pool of Na,K-ATPase into an insoluble pellet (cytoskeletal-associated fraction) and a soluble supernatant by simple differential centrifugation. The redistribution of Na,K-ATPase from pellet to supernatant has been used in several studies as a reproducible marker for ischemic injury of the proximal tubule cell. Using the same technique, the increased Triton X-100 extractability of Na,K-ATPase after renal ischemia was shown to be fully reversible during recovery at a rate dependent on the severity of the insult (3–5).

Recent studies have suggested that HSPs are involved in the restoration of the cytoskeletal anchorage of Na,K-ATPase (4–6). Renal ischemia, *in vivo*, rapidly induces the expression of 25-kD and 70-kD HSPs (4–8). The transient shift of 25-kD HSP, a known actin-binding protein, into the Triton X-100–insoluble pellet strongly suggests interactions with the disrupted cytoskeleton during the postischemic cellular reorganization (6, 9). The 70-kD HSPs, a family of molecular

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chaperons, show a distinct temporal pattern of postischemic cellular localization and intracellular distribution that coincides with the restoration of disrupted cytoskeletal elements during recovery of the proximal tubule cell from ischemia (8). Using functional *in vitro* assays, we recently demonstrated that enhanced 70-kD HSP activity and abundance results in stabilization of Na,K-ATPase in cytoskeletal subfractions (4, 5).

In the present study we sought to determine whether high cellular levels of 25-kD and 70-kD HSPs after pretreatment with renal ischemia (ischemic conditioning) might stabilize Na,K-ATPase *in vivo* during a subsequent ischemic injury. We compared the cellular localization and distribution of Na,K-ATPase after a single and after repeat ischemia in rat renal cortex, and evaluated potential functional interactions between these HSPs and Na,K-ATPase.

## METHODS

The study was approved by the Institutional Animal Study Review Board. All experiments were performed in male Sprague-Dawley rats, weighing 225–300 g, anesthetized by i.p. thiobutabarbital sodium. Temperature was rectally monitored and sustained at normal values on a warming board throughout the anesthesia period. A polyethylene catheter was secured in an external jugular vein, and 5 mL of 0.9% saline was administered followed by an infusion of 1 mL/h. The abdomen was opened by a median incision, and the kidneys were exposed and, if macroscopically normal, carefully dissected. After anticoagulation with 125 IU of heparin, renal ischemia was performed as previously described (4, 5). After the protocol, anesthetized animals were killed by i.v. or intracardial potassium chloride.

***Bilateral ischemia (ischemic conditioning).*** Bilateral renal ischemia was accomplished by selective occlusion of the right renal artery and aorta just proximal to the left renal artery. After 45 min the clamps were removed, and reperfusion was visually confirmed. The abdominal incision was sutured, and the rats were allowed free access to food and water after recovery from anesthesia.

***Unilateral ischemia (ischemic injury).*** Both preconditioned (18 h after bilateral ischemia) and nonpretreated rats were subjected to unilateral renal ischemia. The left kidney was removed immediately after induction of anesthesia, and the left renal pedicle was ligated to prevent exsanguination. Contralateral renal ischemia was accomplished by selective occlusion of the right renal artery. After 45 min the clamp was removed from the right kidney, and reperfusion was visually confirmed. After reflow of 15 min the right kidney was rapidly removed.

***Triton X-100 extraction.*** Kidneys were decapsulated immediately after harvest on ice, and the renal cortex was homogenized in chilled extraction buffer (300–500 mg tissue in 15 mL) containing 0.1% Triton X-100, 60 mM 1,4-piperazinediethanesulfonic acid, 2 mM 1,2-cyclohexylenedinitriloltriacetic acid, 1 mM EDTA, 1 mM EGTA, 100 mM NaCl, 0.5 mM phenylmethylsulfonyl fluoride, 0.75 mg/L leupeptin, and 0.1 mM DTT using a Potter-Elvehjem homogenizer (Bellco Glass Co., Vineland, NJ, U.S.A.). The homogenate was centrifuged within 10 min at 35,000 × *g* for 14 min at 4°C to separate the Triton X-100–soluble protein fraction from the insoluble cytoskeletal fraction. Protein fractions were saved at −70°C until further analysis.

In three additional experiments, 100-μL aliquots of isolated cytoskeletal pellet from renal cortex obtained either after single or after repeat renal ischemia were thawed on ice in 200 μL of extraction buffer. In parallel, 100-μL aliquots of cytoskeletal pellets isolated after repeat renal ischemia were incubated in 200 μL of buffer with 50 μg of anti 25-kD HSP antibody, with 50 μg of anti 70-kD HSP antibody, or with both antibodies (Stressgen Biotechnologies Corp., Victoria, BC, Canada). These mixtures were resuspended and incubated for 20 min in room temperature. Differential centrifugation was then repeated at 35,000 × *g* for 14 min at 4°C. Supernatants, containing the dissociated proteins, were saved at −70°C until further analysis.

***Western blot analysis.*** Protein concentrations were assessed in duplicate by Bradford analysis. Protein samples were electrophoresed through SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose (4, 5). Membranes were incubated with the primary antibodies against 70-kD HSP (Sigma Chemical Co., St. Louis, MO, U.S.A.), 25-kD HSP (SPA801; Stressgen Biotechnologies), and alpha-subunit of Na,K-ATPase (Upstate Biotechnology, Lake Placid, NY, U.S.A.). Detection was accomplished with secondary antibodies (Sigma Chemical Co.) and ECL using ECL Western blotting analysis system and protocols (Renaissance; PerkinElmer Life Science Products, Boston, MA, U.S.A.).

Densitometric analysis was performed by image analysis software (Image Master Analysis Software; Amersham Pharmacia Biotech, Uppsala, Sweden). For analysis of Western blots, calibration curves were constructed to determine the linearity of the ECL detection system using serial loading of cellular protein fractions. Light emission was proportional to protein loading in the range of 3.75 to 15 μg of a given sample, indicating that 4-fold changes were within the linear range.

***Cellular distribution.*** As previous studies have shown that approximately two thirds of total cellular proteins are recovered in the Triton X-100–soluble fraction, the insoluble pellet was resuspended in half the volume of the original homogenate, resulting in approximately equal protein concentrations in both cellular fractions (approximately 4–6 μg/μL) (4–6). Routine use of protein loading of 7.5 μg was sufficient for analysis of HSP distribution between the soluble and insoluble fraction and for changes of Na,K-ATPase distribution. Induction of HSPs was densitometrically assessed after differential protein loading, comparing 15 μg of nonpretreated samples with 3.75 μg of pretreated samples. One more dilution (20 μg *versus* 2 μg) was added for 70-kD HSP induction.

Specific immunodensitometric signals at three different exposures in the linear range of the protein-to-signal intensity relationship were determined from nine rats in each experimental group and compared with an internal standard (for HSP, pooled ischemia-conditioned cortex; for Na,K-ATPase, pooled nonpretreated cortex). Final data for Na,K-ATPase and HSP distribution between soluble and insoluble cellular fractions and HSP induction were individually computed for each renal

cortex after correction for protein distribution between the different cellular fractions after Triton X-100 extraction.

***Immunocytochemical studies.*** A separate set of animals underwent the same conditioning and ischemic injury along with parallel controls. Renal cortical slices of approximately 2.0 mm were incubated in paraformaldehyde fixative. The fixed slices were embedded in paraffin and sectioned at 4 $\mu$m. After blocking of nonspecific binding sites, the identical primary antibodies to Na,K-ATPase as in Western blot analysis were applied in PBS 0.5% BSA for a total of 1 h. After repeated washes, the sections were then exposed to antimouse MAb conjugated to horseradish peroxidase in an equivalent procedure to the primary antibody. The sections were evaluated on a Zeiss microscope (Axiophot, Zeiss, Germany).

***Data analysis.*** ANOVA or Wilcoxon signed-rank test, as appropriate, was used. Values for each reflow interval were compared with their respective controls and considered to be significantly different if $p < 0.05$. Changes are expressed as mean and 95% confidence intervals.

## RESULTS

In the first part of this study we analyzed whether ischemic conditioning results in increased levels of cytoskeletally associated 25-kD and 70-kD HSPs in rat renal cortex after repeat renal ischemia (Figs. 1 and 2). In nonpretreated renal cortex, unilateral ischemia resulted in a significant translocation of 25-kD and 70-kD HSPs into the cytoskeletal cellular fraction, shifting the ratio of Triton X-100–soluble to –insoluble from 4.2 to 1.2 for 25-kD HSP, and from 6.3 to 1.4 for 70-kD HSP. Pretreatment with bilateral renal ischemia resulted in a significantly increased abundance of both HSPs at 18 h of recovery, the majority detected in the Triton X-100–soluble cellular fraction. At that time, repeat renal ischemia again resulted in a significant translocation of HSPs into the cytoskeletal pellet, shifting the ratio of Triton X-100–soluble to –insoluble from 5.1 to 2.1 for 25-kD HSP, and from 8.1 to 2.5 for 70-kD HSP.

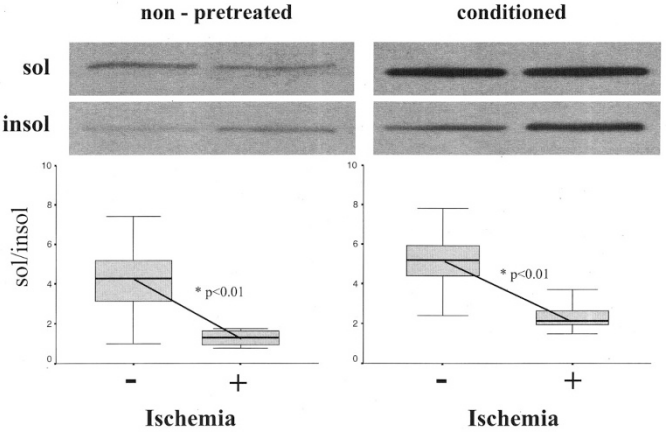


**Figure 1.** Representative immunoblot and densitometry ($n = 9$) of Triton X-100–soluble and –insoluble 25-kD HSPs in rat renal cortex. In nonpretreated animals (*left*), unilateral renal ischemia (+) resulted in a shift of 25-kD HSPs to the cytoskeletal pellet. In animals with ischemic conditioning (*right*), the 25-kD HSPs were markedly elevated. Repeat ischemia (+) resulted again in a shift of 25-kD HSPs to the cytoskeletal pellet.

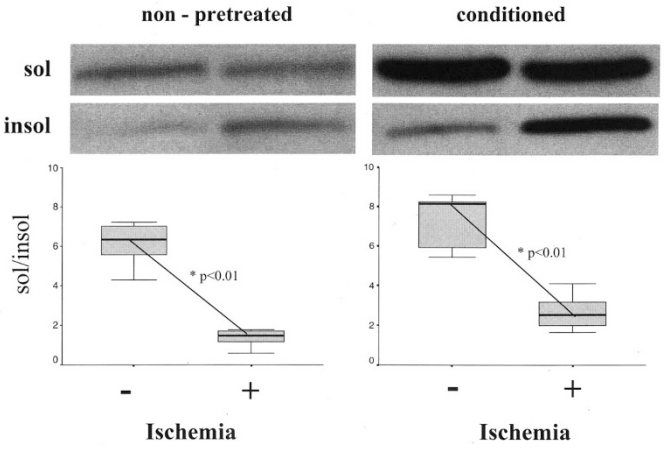


**Figure 2.** Representative immunoblot and densitometry ($n = 9$) of Triton X-100–soluble and –insoluble 70-kD HSPs in rat renal cortex. In nonpretreated animals (*left*), unilateral renal ischemia (+) resulted in a shift of 70-kD HSPs to the cytoskeletal pellet. In animals with ischemic conditioning (*right*), the 70-kD HSPs were markedly elevated. Repeat ischemia (+) resulted again in a shift of 70-kD HSPs to the cytoskeletal pellet.

In combination with the marked induction of total cellular HSPs after ischemic pretreatment (7.5-fold for 25-kD HSP and 19.5-fold for 70-kD HSP) this cellular redistribution after repeat ischemia resulted in high levels of cytoskeletal-associated 25-kD and 70-kD HSPs in conditioned rat renal cortex.

In the second part of the study, we analyzed whether ischemic conditioning stabilizes the cytoskeletal association of Na,K-ATPase in rat renal cortex after repeat renal ischemia (Figs. 3 and 4). In nonpretreated animals, renal ischemia resulted in a marked increase of Triton X-100–extractable Na,K-ATPase at 15 min of reflow (1.8-fold increase (1.4–2.2; $p < 0.01$). In conditioned animals (at 18 h of reflow) Triton

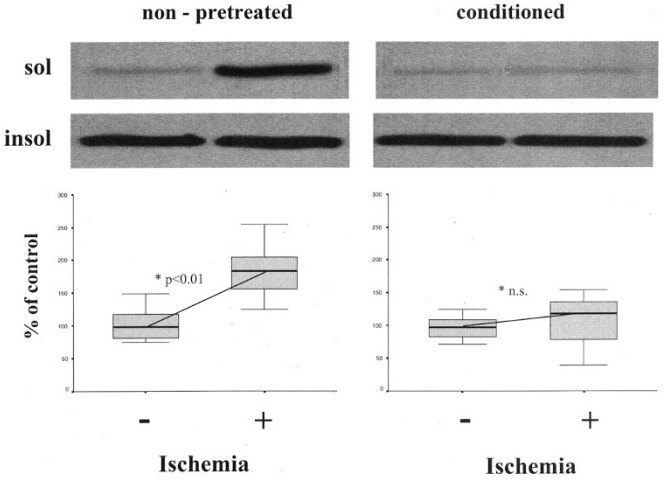


**Figure 3.** Representative immunoblot and densitometry ($n = 9$) of Triton X-100–soluble and –insoluble Na,K-ATPase in rat renal cortex. In nonpretreated animals (*left*), unilateral renal ischemia (+) resulted in a marked increase of Triton X-100–soluble Na,K-ATPase. In animals with ischemic conditioning (*right*), Triton X-100–soluble Na,K-ATPase had returned to basal levels. After repeat ischemia there was no significant increase in Triton X-100 extractability of Na,K-ATPase (+). Data are expressed as percentage of the specific signal from pooled renal cortex from nonpretreated rats.

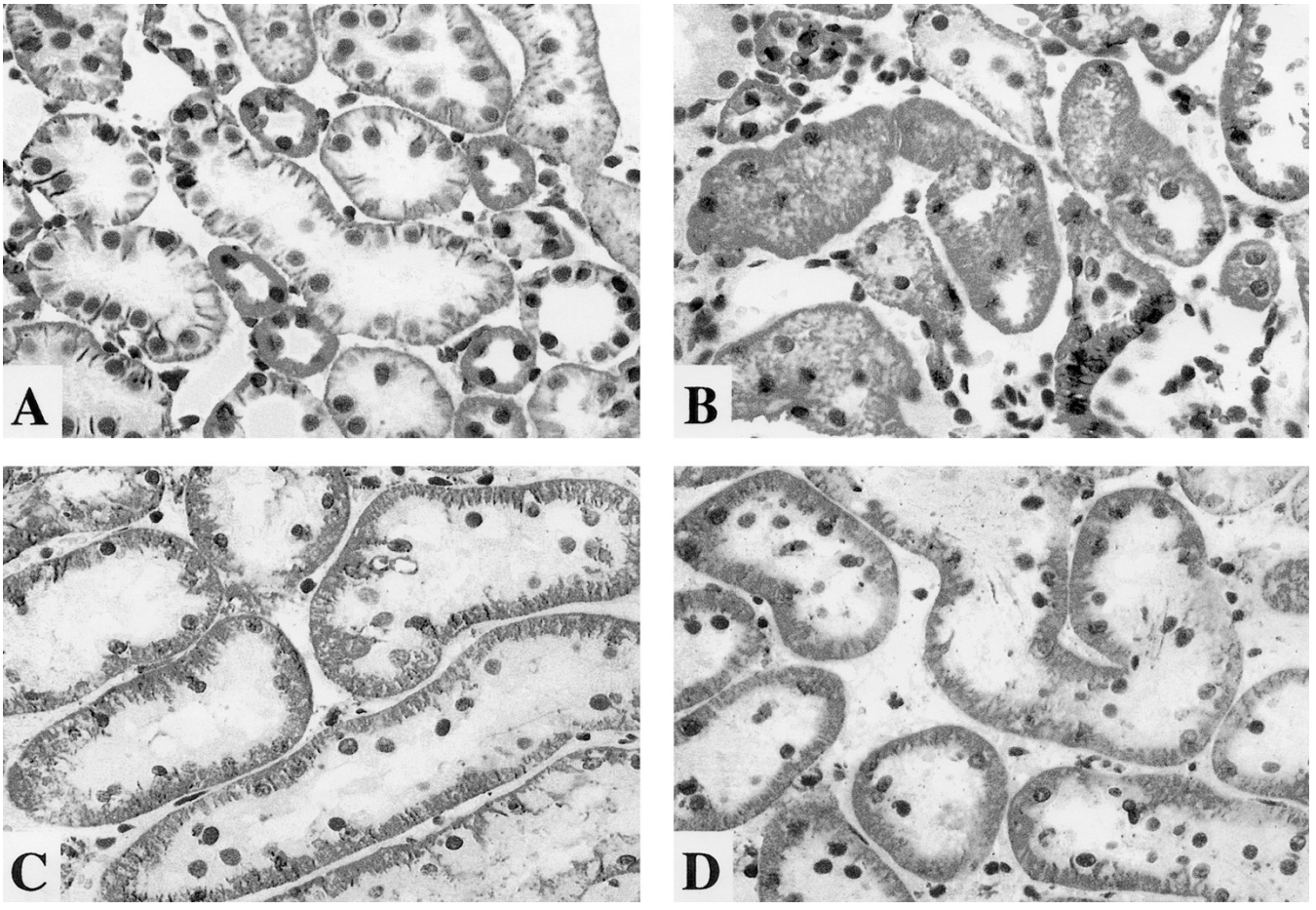


**Figure 4.** The pattern of immunolocalization of Na,K-ATPase was compared in proximal tubular cells of nonpretreated rats (*A, B*) and of ischemia-conditioned rats (*C, D*) between renal cortex harvested before (*A, C*) or after (*B, D*) ischemia. The staining patterns suggest that the Na,K-ATPase localization is stabilized during repeat ischemia in conditioned rat renal cortex.

X-100–extractable Na,K-ATPase had returned to baseline levels. Repeat renal ischemia resulted in no significant increase in Triton X-100 extractability of Na,K-ATPase (0.8–1.4). Reduction in Triton X-100–insoluble cytoskeletal Na,K-ATPase did not reach statistical significance after single or repeat ischemia (Fig. 3).

This stabilization of Na,K-ATPase localization in conditioned renal cortex was also found by immunohistochemistry (Fig. 4). In proximal tubule cells of nonpretreated rat renal cortex, renal ischemia resulted in obvious dislocation of Na,K-ATPase from the basolateral domain into a diffuse cytoplasmic pattern with an overall decreased signal. In proximal tubule cells of conditioned renal cortex (at 18 h of reflow), Na,K-ATPase had reaccumulated in the basolateral domain. Repeat ischemia resulted in no discernible disruption of this staining pattern. The pattern of tubule Na,K-ATPase distribution remained unchanged in conditioned renal cortex after the second ischemic injury, when compared with the pattern in the contralateral conditioned kidneys, which did not undergo repeat ischemia.

In the final part of this study, we analyzed for functional interactions between 25-kD and 70-kD HSPs and stabilization of Na,K-ATPase in cytoskeletal fractions of rat renal cortex isolated after ischemic conditioning (Fig. 5). The differential

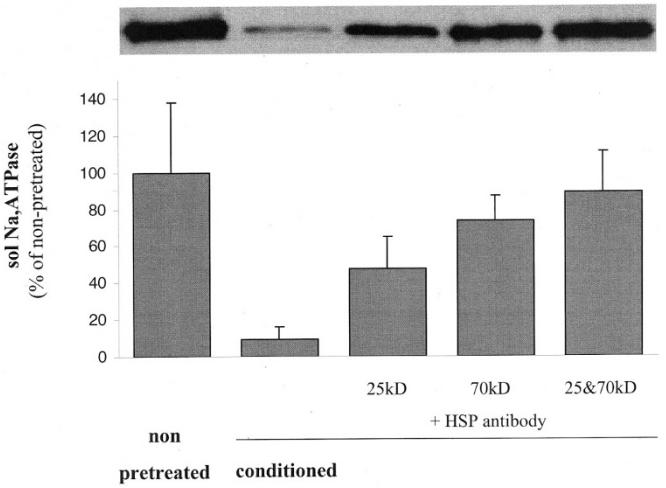


**Figure 5.** Representative immunoblot and densitometry of differential Triton X-100 extractability of Na,K-ATPase from isolated cytoskeletal associated fractions ($n = 3$). Isolated cytoskeletal pellets obtained from rat renal cortex treated either by single (nonpretreated) or by repeat renal ischemia (conditioned) were incubated with anti-25-kD or anti-70-kD HSP antibody, or both. After the incubation interval, repeat Triton X-100 extraction was performed. Cytoprotective effects of ischemic conditioning (as demonstrated by stabilization of Na,K-ATPase in the cytoskeletal pellet isolated after repeat renal ischemia) were reversed by addition of anti-HSP antibodies.

extractability of Na,K-ATPase from cytoskeletal fractions isolated from renal cortex either after single or after repeat ischemia was reproduced in a functional *in vitro* assay. Parallel incubation and repeat Triton X-100 extraction of isolated cytoskeletal protein fractions demonstrated significant stabilization of Na,K-ATPase in the conditioned cytoskeletal fraction.

We then found direct evidence for functional interactions between the HSPs and the cytoskeletal fraction associated with stabilization of Na,K-ATPase in this fraction during ischemic conditioning. Addition of antibodies against 25-kD or 70-kD HSPs attenuated stabilization of Na,K-ATPase in the cytoskeletal fraction (Fig. 5). Incubation of conditioned cytoskeletal pellets with extraction buffer enriched with antibodies against 25-kD or 70-kD HSPs resulted in markedly increased Triton X-100 extractability of Na,K-ATPase. The effect of adding antibody against both HSPs was additive and resulted in complete elimination of preserved Na,K-ATPase association with the cytoskeletal fraction in the preconditioned pellets.

## DISCUSSION

Our study demonstrates that *in vivo* ischemic conditioning prevents dissociation of Na,K-ATPase from its cytoskeletal anchorage in rat renal cortex after repeat ischemia. This *in vivo* stabilization of Na,K-ATPase was associated with marked induction and cytoskeletal redistribution of 25-kD and 70-kD HSPs in conditioned renal cortex. Incubation of isolated cytoskeletal fractions of conditioned renal cortex with blocking antibodies against 25-kD or 70-kD HSPs abolished this stabilization of Na,K-ATPase *in vitro*.

Renal ischemia is known to cause transient disruption of the cellular localization and distribution of Na,K-ATPase in the proximal tubular cell after nonlethal injury (3, 10). For Na,K-ATPase to be translocated from its basolateral membrane domain, it must first be detached from its cytoskeletal anchorage, which has been defined by Triton X-100 extractability (2, 3). In agreement with previous reports, this study shows that Triton X-100–extractable Na,K-ATPase was increased after single renal ischemia and had returned to basal levels in renal cortex after bilateral ischemia and 18 h of reflow. This restoration of the cytoskeletal association of Na,K-ATPase has been shown to occur by recycling of displaced elements rather than by their increased biosynthesis (1, 10).

The HSPs are ideal candidates for such posttranslational repair mechanisms (11, 12). 25-kD HSPs are known to bind to the actin cytoskeleton, and 70-kD HSPs bind to hydrophobic sites of disrupted proteins (12). From these established functions of 25-kD and 70-kD HSPs, one would predict a transiently increased affinity of both HSPs for the Triton X-100–insoluble cellular fraction of rat renal cortex isolated after ischemic injury. In this study, we confirm that renal ischemia rapidly induces the elaboration of 25-kD and 70-kD HSPs (4, 6–8). Our data further demonstrate that in addition to 25-kD HSPs, 70-kD HSPs also translocate into the cytoskeletal cellular fraction after renal ischemia (6). Moreover, these results extend the role of 25-kD and 70-kD HSPs from postischemic repair to potential cytoprotection against repeat ischemia, as not only single but also repeat renal ischemia resulted in a marked cytoskeletal shift of both HSPs. As ischemic conditioning potently increased the expression levels of these inducible HSPs but did not alter their pattern of postischemic redistribution, repeat ischemia resulted in high levels of 25-kD and 70-kD HSPs in the cytoskeletal fraction of cellular proteins.

This fraction is known to contain the actin cytoskeleton, Na,K-ATPase, and other cytoskeletal-associated proteins that are able to bind to each other (3–7). Stabilization by capping proteins like 25-kD HSP or molecular chaperons such as 70-kD HSP may therefore result in improved binding and stabilization of the cytoskeletal anchorage of Na,K-ATPase (4–6, 12). Our data support this hypothesis as repeat renal ischemia did not cause a repeat increase of Triton X-100–extractable Na,K-ATPase in the conditioned renal cortex. Furthermore, the immunohistochemical findings are consistent with the detergent extractability data. In agreement with previous studies, the cellular distribution of Na,K-ATPase was basolateral in proximal tubular cells in renal cortex harvested from control animals and was more diffusely distributed throughout the cell after single renal ischemia (10). In renal cortex harvested after 18 h of reflow (ischemic conditioning) the tubules display some typical postischemic changes including cellular dropout with loss of tubular integrity; however, the remaining proximal tubule cells show that basolateral localization of Na,K-ATPase is restored at this interval. Repeat ischemia after such ischemic conditioning, at high cellular abundance of 25-kD and 70-kD HSPs, resulted in no repeat disruption of the basolateral distribution of Na,K-ATPase. Using colocalization studies, we recently found close physical proximity between 25-kD HSPs and disrupted (DNAse-reactive) actin fragments after renal ischemia (6). Similar postischemic colocalization was found between the molecular chaperon 70-kD HSP and disrupted, displaced Na,K-ATPase (13). These temporal and spatial associations suggest that *in vivo* stabilization of Na,K-ATPase after ischemic conditioning might at least in part be mediated by high levels of cytoskeletally associated 25-kD and 70-kD HSPs.

Using a similar *in vivo* protocol Zager and Baltes (14) have previously described that rat kidneys exhibited comparable morphologic injury after single and repeat ischemia, and that repeat ischemia did not prolong overall postischemic recovery of renal function. In contrast, heat pretreatment did not protect morphology or global function against experimental renal ischemia in isolated perfused kidneys or proximal tubules, despite comparable induction of 70-kD HSP (15, 16). Interestingly, however, heat-conditioned kidneys demonstrated significant preservation of sodium reabsorption (15). As the authors did not assess cellular localization or distribution of Na,K-ATPase in proximal tubule cells, it could be that the heat pretreatment resulted in an unappreciated preservation of Na,K-ATPase polarity and function, thus preserving sodium reabsorptive ability. HSP-mediated stabilization of the cytoskeletal anchorage and localization of Na,K-ATPase could be the underlying mechanism. Consistent with this concept, heat conditioning of cultured renal cells resulted in induction of 70-kD HSPs and preservation of cytoskeletal structure against subsequent injury

by energy depletion (17). Similarly, selective overexpression of 25-kD HSPs protected the actin-based cytoskeleton against disruption by subsequent injurym and transfection of proximal tubular cells with 70-kD HSPs resulted in cytoprotection against heat (9, 18). Thus, findings from other *in vivo* and *in vitro* systems also corroborate that effects of 25-kD and 70-kD HSPs might mediate cytoprotection conferred by conditioning treatments.

In search of direct evidence for HSP-mediated restoration of the cytoskeletal anchorage of Na,K-ATPase after *in vivo* renal ischemia, we recently have developed a functional *in vitro* repair assay (5). In this system, coincubation of cytoskeletal and associated proteins obtained during early reflow after renal ischemia (exhibiting severe injury of the cytoskeletal anchorage of Na,K-ATPase) and noncytoskeletal proteins obtained during later reflow (showing high HSP expression) resulted in specific translocation of 70-kD HSPs from the noncytoskeletal supernatant into the cytoskeletal pellet, functionally associated with dose-dependent stabilization of Na,K-ATPase within this cytoskeletal fraction. This stabilization was reproduced by purified HSP and abolished by anti-HSP antibodies (5). In the present study, we modified this *in vitro* assay to investigate the role of 25-kD and 70-kD HSPs in the stabilization of Na,K-ATPase after ischemic conditioning. Based on our previous findings, we expected that high abundance of HSPs would stabilize whereas blocking of HSPs would disrupt the association of Na,K-ATPase with isolated cytoskeletal fractions. As expected, Na,K-ATPase was stabilized in cytoskeletal fractions of conditioned renal cortex (isolated at high abundance of HSPs). This stabilization was almost completely abolished *in vitro* by incubation with antibodies simultaneously against 25-kD and 70-kD HSPs. Incubation with antibody against an individual HSP attenuated the effect. As the effects of each antibody were additive, the findings are consistent with different, but complementary, functions of the actin-binding 25-kD HSP and the molecular chaperon 70-kD HSP. These *in vitro* results provide direct evidence for essential roles of both HSPs in the cytoprotection against repeat renal ischemia after ischemic conditioning.

Taken together, this study confirms the concordant induction of the 25-kD and 70-kD HSPs after renal ischemia. It further shows increased association of these HSPs with the cytoskeletal fraction after single or repeat renal ischemia. In contrast to single ischemia, repeat ischemia at high cellular abundance of 25-kD and 70-kD HSPs did not result in disruption of the cellular distribution or localization of Na,K-ATPase. The stabilization of Na,K-ATPase interactions with the cytoskeleton could be reversed *in vitro* by antibodies against 25-kD and 70-kD HSPs. This study thus supports the concept that ischemic conditioning protects the integrity of the cytoskeletal anchorage of Na,K-ATPase after repeat renal ischemia by HSP-mediated mechanisms.

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