Kidney International, Vol. 54, Suppl. 67 (1998), pp. S-174–S-176

Effect of ischemia on localization of heat shock protein 25 in kidney

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Effect of ischemia on localization of heat shock protein 25 in kidney. The effects of renal ischemia on the intracellular distribution of the low-molecular weight heat shock protein (HSP)25 were examined using immunofluorescence microscopy. In all kidney zones, ischemia decreased HSP25 in the supernatant of the tissue homogenates and increased it in the pellet fraction (containing mainly nuclei and cytoskeletal components). This was associated with disappearance of HSP25 staining from the brush border of proximal convoluted tubule (PCT) cells. Because no nuclear staining of cortical tubule cells was apparent either in control or ischemic kidneys, ischemia seems to cause a closer association of HSP25 with cytoskeletal components. HSP25 probably participates in the postischemic restructuring of the cytoskeleton of PCT cells.

Interruption of renal blood flow leads to intracellular redistribution of the low-molecular weight heat shock protein 25 (HSP25) in the cells of the injured kidney [1]. It is unknown whether this intracellular redistribution is caused by HSP25 moving from the cytosol into the nucleus or/and by a closer association of this HSP with cytoskeletal structures such as actin. We thus characterized in greater detail the ischemia-induced intracellular redistribution of HSP25.

METHODS

Male Wistar rats (Charles River, Sulzfeld, Germany) kept on a standard diet (Alma, Kempten, Germany) were anesthetized by i.p. injection of thiobutabarbitone sodium (Inactin, 100 to 120 mg/kg body weight; Byk-Gulden, Konstanz, Germany). The surgical protocol and preparation of the left kidney have been described elsewhere [1]. Ischemia of the left kidney (60 minutes) was achieved by occluding the left renal artery by a weak clip. Both kidneys were harvested immediately after the 60 minutes of ischemia and were frozen in propane/isopentane (3:1, −196°C). The contralateral nonischemic kidney served as the control.

Key words: proximal convoluted tubule, van Willebrand's factor, cytoskeleton, actin, microfilaments in endothelial cells, cytoskeletal restructuring.

For indirect immunofluorescence microscopy, 4-μm sections were cut at −20°C (CM3050 cryotome; Leica, Nussloch, Germany) and were fixed immediately in 4% paraformaldehyde. The sections were heated in a citric acid buffer (10 mM, pH 6) in a microwave oven for 10 minutes. Endogenous avidin binding was blocked with an avidin-biotin blocking kit (Zymed, San Francisco, CA, USA) and nonspecific antibody binding sites with 20% normal goat serum (Life Technologies, Eggenstein, Germany) and 0.01% Tween-20. For immunodetection, sections were incubated with a HSP25-specific antiserum [SPA-801; Stress Gen, Victoria, Canada; 1:500 dilution in phosphate-buffered saline (PBS)/goat serum/Tween-20; 1 hour], washed in PBS, and incubated with a biotin-SP-conjugated goat antirabbit IgG (Dianova, Hamburg, Germany; 1:500 dilution in PBS/goat serum/Tween-20). The sections were rewashed and exposed to rhodamine (TRITC)-conjugated streptavidin (Dianova; 1:400 dilution in PBS/goat serum/Tween-20; 30 minutes) in a light-proof container. Negative controls for each antibody were processed in parallel by

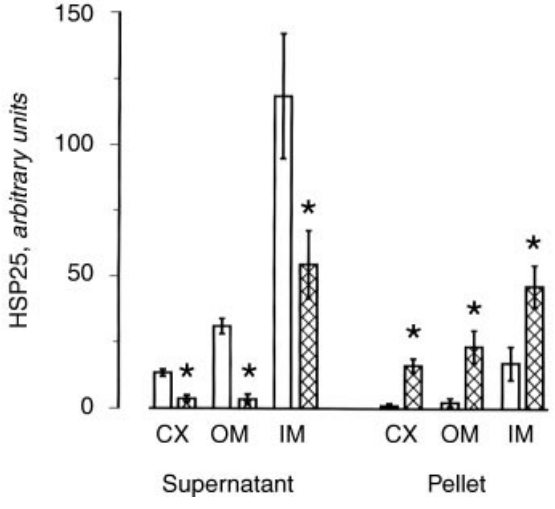

Fig. 1. Extractable (supernatant fraction, left) and nonextractable (pellet fraction, right) heat shock protein 25 (HSP25) in cortex (CX), outer medulla (OM), and inner medulla (IM) in ischemic left kidneys (▨) and in nonischemic right kidneys (controls, □; $N = 4$). Data is taken from [1]; means ± SEM. $^*P < 0.05$ versus corresponding control.

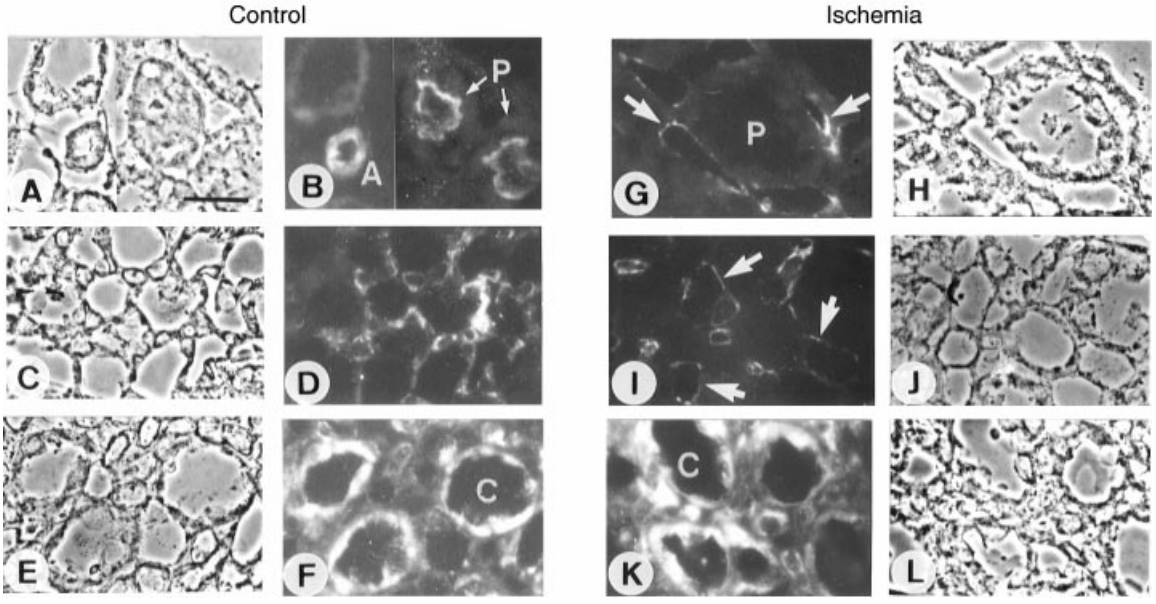

Fig. 2. Intracellular localization of heat shock protein (HSP)25 in control (left, *A—F*) and ischemic (right, *G—L*) kidneys. Phase contrast (A, C, E, H, J, L) and immunofluorescence (B, D, F, G, I, K) microscopy on paraformaldehyde-fixed cryosections treated with antiserum directed against HSP25, biotin-conjugated IgG, and rhodamine-conjugated avidin (see the **Methods** section). Sections are from cortex (A, B, G, H), outer medulla (C, D, I, J), and inner medulla (E, F, K, L). Larger arrows indicate small, circular, HSP25-positive structures. Bar in A represents 25 μm. Abbreviations are: P (with smaller arrows), proximal tubule; A, arteriole; C, collecting duct.

omitting the primary antibody. Finally, the sections were mounted under cover slips with FluorSave TN Reagent (Calbioch, Bad Soden, Germany) and were stored at 4°C in the dark until visualization in an inverted microscope (IM35; Zeiss, Oberkochen, Germany). A separate series of methanol-fixed (−20°C, 5 minutes) cryosections was used for double labeling with a monoclonal mouse antibody (Boehringer, Mannheim, Germany) against von Willebrand's factor (vWF) and the above antiserum against HSP25. For vWF staining, a FITC-conjugated goat anti-mouse antibody (DAKO, Hamburg, Germany) was used as secondary antibody (primary and secondary antibody dilution 1:10). After vWF staining, the sections were stained for HSP25 as mentioned earlier here. Stains were visualized with filter combinations appropriate for monitoring fluorescence of FITC or rhodamine.

RESULTS AND DISCUSSION

Ischemia (60 minutes) induces a significant rise in non-extractable HSP25 in all kidney zones (Fig. 1) [1]. In the cortex of control kidneys, staining for HSP25 was conspicuous in the brush border region of the proximal convoluted tubule (PCT) and vascular structures (Fig. 2B). Immediately following ischemia, HSP25 was no longer detectable in the PCT brush border (Fig. 2G). HSP25 was not detectable in nuclei of cortical tubule segments in either control or ischemic kidneys. These results suggest that, in proximal tubule cells, ischemia causes relocalization of HSP25 from the brush border region to a nonnuclear cell comparment. Taking into account the ischemia-induced dispersion of stainable actin in PCT cells (restricted to the apical pole in control cells, dispersed throughout the cytoplasm after ischemia [2]) and the observations that HSP25 interacts with actin [3, 4] and that the nonextractable fraction of HSP25 increases during ischemia (Fig. 1), it is likely that ischemia provokes a closer association of low-molecular weight HSP with cytoskeletal structures, that is, a decreased HSP25 solubility. Low-molecular weight HSPs may thus participate in the reorganization of the cortical actin network observed in proximal tubule cells after transient ischemia [2].

In the ischemic kidney, distinct staining of circular structures close to cortical tubule segments was noted (Fig. 2G). Because in methanol-fixed tissue sections many of these HSP25-positive peritubular structures were also positive for vWF (data not shown), at least some of these peritubular HSP25-positive structures are probably vascular components. In the outer medulla of controls, HSP25-positive structures (Fig. 2D) were grouped between areas without noticeable HSP25 staining. Some of the HSP25-positive structures stained also for vWF (data not shown). This staining pattern was largely unchanged after ischemia, except for the appearance of additional, small, circular, HSP25-positive structures (Fig. 2I) in areas in which, under control conditions, staining for HSP25 was barely noticeable. These HSP25-positive structures probably represent vessels, as revealed by phase contrast microscopy and vWF staining. The fact that endothelial cells naturally express

high levels of the small HSP and that this HSP is involved in the oxidative stress-induced actin reorganization in these cells [5] supports the view that HSP25 may participate in the microfilament response to ischemic stress also in endothelial cells of the kidney. As also shown in Figure 2, ischemia did not noticeably affect the intense HSP25 staining of collecting duct cells in the inner medulla. Whether the ischemia-induced increase in nonextractable HSP25 in this kidney zone (Fig. 1) is due to closer association of HSP25 with cytoskeletal or other proteins or to multioligomerization of this HSP remains to be elucidated.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (Be 963/4-4). The presented material is part of A. Schober's M.D. thesis.

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