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Upregulation of Heat Shock Proteins in the Kidney in Hypertension

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The present study was undertaken to determine the regulation of heat shock proteins (HSP) in the kidney in hypertension. Two-kidney, one clip (2K1C) or deoxycorticosterone acetate (DOCA)-salt hypertension was induced in male Sprague-Dawley rats. At weeks 1 and 4 after inducing the hypertension, the expression of HSP70, HSP32 and HSP25 was determined in the kidney by Western blot analysis. In 2K1C hypertension, the expression of HSP70, HSP32 and HSP25 was increased in the clipped kidney at both weeks 1 and 4. However, in the contralateral kidney, their expression was not significantly altered at week 1, but increased at week 4. In DOCA-salt hypertension, the expression of HSP remained unaltered in the remnant kidney at week 1, but significantly increased at week 4. These results indicate that HSP are differentially regulated in the kidney according to the duration and the model of hypertension.

Key Words: Heat shock proteins, Two-kidney, One clip hypertension, Deoxycorticosterone acetate-salt hypertension, Kidney

INTRODUCTION

Heat shock proteins (HSP) may be induced in response to a variety of stresses, including hypertension. The cellular expression of major HSP gene is increased in response to heat exposure in hypertensive animals and humans (Hamet et al, 1990). Their overexpression has been related with a protective role in various pathophysiological states. For instance, an induction of HSP70 protects mesangial cells against oxidative injury (Chen et al, 1999), and an induction of heme oxygenase (HO)-1 in the heart attenuates cardiac hypertrophy in load-independent mechanism in genetically hypertensive rats (Seki et al, 1999). More recent study also demonstrated an increased coronary HO-1 expression associated with increased vasoconstrictor responsiveness to an inhibitor of HO-1 in Dahl/Rapp salt-sensitive hypertension, which may provide cardioprotection by promoting coronary vasodilation (Johnson et al, 2004).

In the kidney, the expression of HSP70 and HSP25 is increased in angiotensin II-infused rats (Ishizaka et al, 2002). Although the regulation of HSP may be altered in the kidney in hypertension, its pathophysiological implication has not been widely explored in various models of hypertension. The present study was undertaken to examine the regulation of HSP in the kidney in different models of hypertension. Rats were made either two-kidney, one clip (2K1C) or deoxycorticosterone acetate (DOCA)-salt hypertensive, and their expression of HSP70, HSP32, and HSP25 was determined in the kidney.

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METHODS

Animals

Male Sprague-Dawley rats, weighing 180~200 g, were used. They were kept in accordance with the Institutional Guidelines of Experimental Animal Care and Use. 2K1C hypertension was made by constriction of the left renal artery with a silver clip having an internal gap of 0.25 mm under anesthesia with ketamine (50 mg/kg, *IP*). Control group was without clipping the renal artery. Both experimental and control groups of rats were then kept for 1 or 4 weeks while given food and tap water ad libitum. To develop DOCA-salt hypertension, the rats were divided into two groups one week after the unilateral nephrectomy: one group was subcutaneously implanted with silastic strips impregnated with DOCA (200 mg/kg) and given 0.9% saline to drink, and the other was given 0.9% saline to drink without implantation of DOCA strips. They were used 1 or 4 weeks thereafter.

On the day of the experiment, systolic blood pressure was measured indirectly at the tail artery in a conscious state. It steadily increased after inducing the hypertension in the experimental group (Table 1).

ABBREVIATIONS: HSP, heat shock proteins; 2K1C, Two-kidney, one clip; DOCA, deoxycorticosterone acetate

Western blot analysis

After measuring the blood pressure, the animals were decapitated in a conscious state. The kidneys were rapidly removed and kept at −70°C until assayed. They were thawed in ice-cold phosphate-buffered saline, and were homogenized at 3,000 rpm in a solution containing 250 mmol/L sucrose, 1 mmol/L ethylenediamine tetraacetic acid (EDTA), 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and 10 mmol/L Tris-HCl buffer, at pH 7.6. Large tissue debris and nuclear fragments were removed by two low speed spins in succession (1,000 g, 10 min; 10,000 g, 5 min). The supernatant was then collected for protein blotting. The protein concentration was determined using bicinchonic acid assay kit (Bio-Rad, Hercules, CA, USA).

Protein samples were loaded and electrophoretically size-separated with a discontinuous system consisting of 12.5% or 10% polyacrylamide resolving gel and 5% polyacrylamide stacking gel. The proteins were then electrophoretically transferred to a nitrocellulose membrane at 40 V for 3 hr. The membranes were washed in Tris-based saline buffer (pH 7.4) containing 0.1% Tween-20 (TBST; Amresco; Solon, OH, USA), and blocked with 5% nonfat milk in TBST for 1 hr. They were then incubated with affinity-purified anti-mouse monoclonal antibodies against HSP70 (1 : 2,000), anti-mouse polyclonal antibodies against HSP32 (1 : 5000), or anti-rabbit polyclonal antibodies against HSP25 (1 : 2,000) in 0.2% nonfat milk/TBST for 2~3 hr at room temperature. HSP antibodies were purchased from StressGen (Victoria, BC, Canada). The membranes were again incubated with a horseradish peroxidase-labeled goat anti-rabbit IgG (1 : 2,000) in 2% nonfat milk/TBST for 1 hr. The bound antibody was detected by enhanced chemiluminescence (Amersham; Little Chalfont, Buckinghamshire, UK) on hyperfilm. Relative protein levels were determined by analyzing the signals of autoradiograms using the transmitter scanning videodensitometer (Bioneer; Cheongwon, Korea).

Table 1. Systolic blood pressure in two-kidney, one clip and deoxycorticosterone acetate-salt rats

	Control	Experimental
2K1C		
Week 1	121±3	152±5**
Week 4	123±5	180±6**
DOCA-salt		
Week 1	122±4	139±5**
Week 4	128±4	167±6**

Numbers of rats were 6 each. *P<0.05, **P<0.01; compared with control.

Statistics

All data are expressed as mean±SEM. Differences between the experimental and control groups were assessed by the unpaired t-test.

RESULTS

2K1C Hypertension

HSP70, HSP32, and HSP25 were recognized at ~73, ~35,

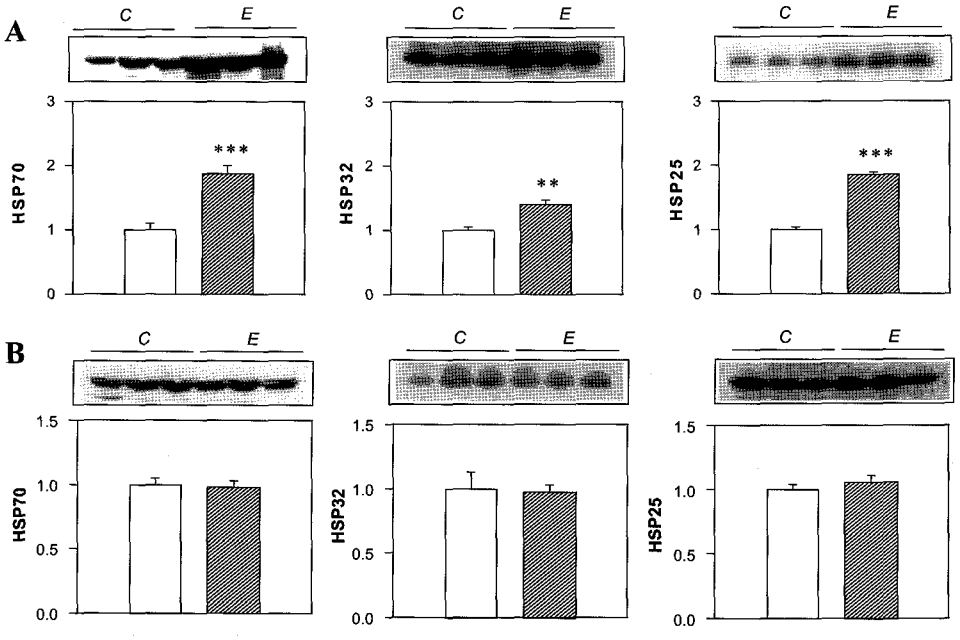

Fig. 1. Expression of HSP70, HSP32, and HSP25 in the clipped (A) and the contralateral kidneys (B) at week 1. Western blots and their densitometric data are shown. HSP70, HSP32, and HSP25 were recognized at ~73, ~35, and ~24 kDa bands, respectively. Open columns show the control and hatched columns represent the experimental (mean±SEM of 6 experiments). **P<0.01, ***P<0.001; compared with control.

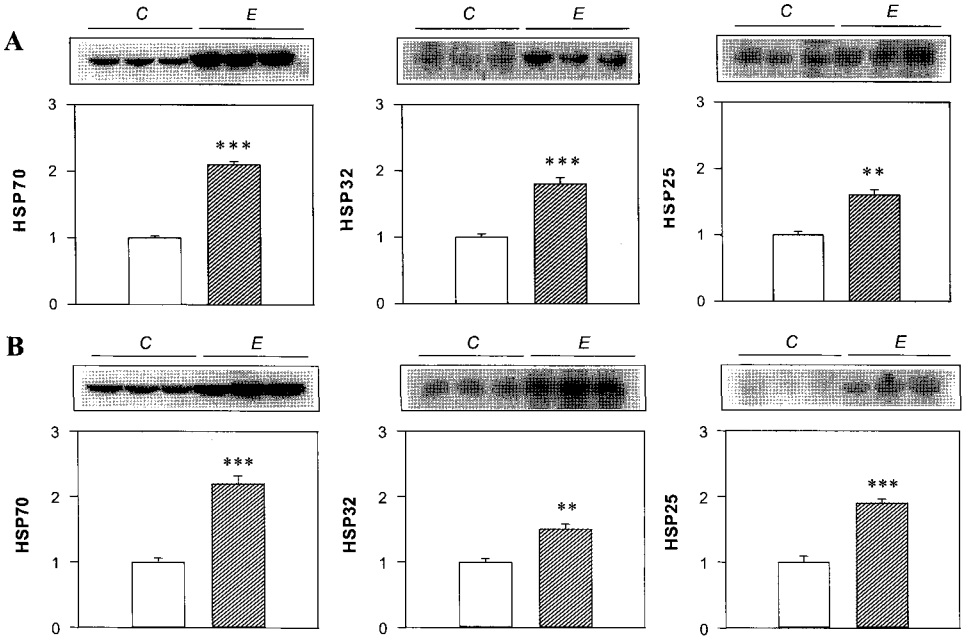

Fig. 2. Expression of HSP70, HSP32, and HSP25 in the clipped (A) and the contralateral kidneys (B) at week 4. Open columns show the control and hatched columns represent the experimental (mean±SEM of 6 experiments). **P<0.01, ***P<0.001; compared with control.

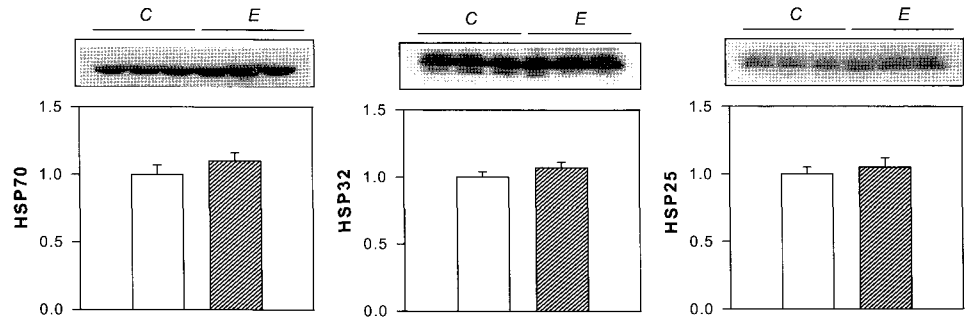

Fig. 3. Expression of HSP70, HSP32, and HSP25 in the remnant kidney in DOCA-salt hypertension at week 1. Open columns show the control and hatched columns represent the experimental (mean±SEM of 6 experiments).

and ~24 kDa bands, respectively. The expression of HSP70, HSP32, and HSP25 was significantly increased in the clipped kidney, while not significantly altered in the contralateral kidney at week 1 (Fig. 1). On the contrary, at week 4, their expression was upregulated both in the clipped and the contralateral kidneys (Fig. 2).

DOCA-Salt Hypertension

In DOCA-salt hypertension, HSP remained unaltered at week 1 (Fig. 3), but increased significantly at week 4 (Fig. 4).

DISCUSSION

In 2K1C rats, the clipped kidney showed an increased expression of different isoforms of HSP at weeks 1 and 4 after inducing the hypertension, while the contralateral kidney showed a delayed increase at week 4 with no significant changes at week 1. An ischemic insult in the kidney has been shown to induce the expression of HSP (Smoyer et al, 2000). In this context, the early increase of HSP in the clipped kidney can be related with an ischemic insult.

On the contrary, the upregulation of HSP in both the clipped and the contralateral kidneys at week 4 may be in line with the previous observation, in which angiotensin II infusion induces the renal expression of HSP70, HSP32 and HSP25 (Ishizaka et al, 2002). An increase of circulating levels of angiotensin II in 2K1C rats may have induced an upregulation of HSP in the clipped and the contralateral kidneys.

In DOCA-salt hypertension, the remnant kidney showed a delayed increase of HSP isoforms: the expression was not

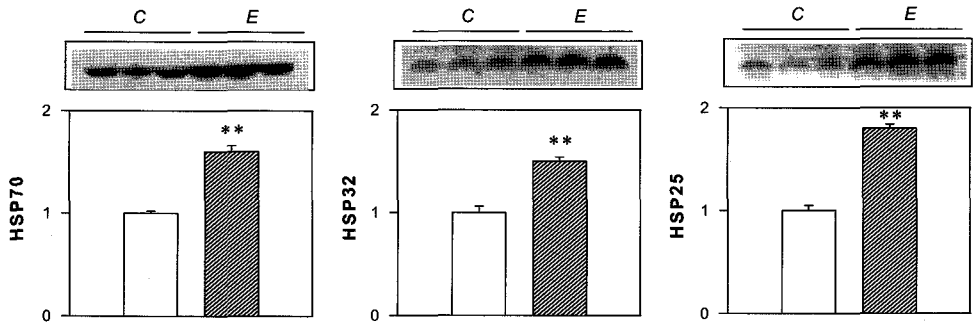

Fig. 4. Expression of HSP70, HSP32, and HSP25 in the remnant kidney in DOCA-salt hypertension at week 4. Open columns show the control and hatched columns represent the experimental (mean ± SEM of 6 experiments). **P < 0.01, compared with control.

significantly altered at week 1, but increased at week 4. Hamet et al (1990) observed an enhanced expression of HSP70 in the kidney after heat exposure of the whole body in genetic hypertension. HSP70 polymorphism has been associated with a blood pressure difference in spontaneously hypertensive rats (Hamet et al, 1992). Hamet et al (1996) also found that the contribution of HSP27 allele to cardiac hypertrophy was independent of blood pressure. A pressor-independent upregulation of renal HSP70 has been also observed by other investigators (Xu et al, 1996). These findings may indicate that an increase of systemic blood pressure per se may not be sufficient to induce HSP. Therefore, it is not certain whether the slow induction of HSP in the contralateral kidney of 2K1C rats and the remaining kidney of DOCA-salt rats was related with the high blood pressure. However, it has been shown that an in vitro translation of aortic RNA from DOCA-salt hypertensive rats does not reveal HSP induction (Kohane et al, 1990). The delayed induction of HSP in the contralateral kidney of 2K1C rats and the remaining kidney of DOCA-salt rats may be related with a prolonged increase of systemic blood pressure and hence hemodynamic stress.

Alterations of HSP may play a protective role against potential tissue damage. Molecular adaptation of vascular endothelial cells to oxidative stress is accompanied by an induction of HSP (Lu et al, 1993). An induction of HSP70 has a protective effect on the vasculature from hemodynamic stress (Xu et al, 1995). The small HSP from human HSP27 and mouse HSP25 form large oligomers which can act as molecular chaperones in vitro, and protect cells from heat shock and oxidative stress when overexpressed (Rogalla et al, 1999). Pre-conditioning of aortic smooth muscle cells through induction of the heat shock response prior to physical injury may be a useful approach to limit aggressive proliferation observed with mechanical revascularization injury (Slepian et al, 1996).

In the kidney, an induction of HO-1/HSP32 ameliorates the tissue injury induced by rhabdomyolysis (Nath et al, 1992). An induction of HSP, including HSP70, HSP25 and HSP32, has been suggested to have a renoprotective effect against angiotensin II-induced injury (Aizawa et al, 2000; Ishizaka et al, 1997; Ishizaka et al, 2002). An overexpression of HSP70 protects LLC-PK1 tubular cells from heat shock (Turman & Rosenfeld, 1999). An upregulation of human HSP72 in renal tubular cells confers resistance against oxidative injury and cisplatin-toxicity (Komatsuda et al, 1999). Conversely, an ablation of HO-1 exacerbates the renal tubular injury induced by cisplatin (Shiraishi et al, 2000). The upregulation of HSP in 2K1C and DOCA-salt rats may also play a role in the renoprotective action in hypertension.

On the other hand, hydralazine blocked the upregulation of HSP32, but failed to block the upregulation of HSP70 or HSP25 in angiotensin II-infused rats (Ishizaka et al, 2002). This finding may indicate that different mechanisms are responsible for the regulation of different isoforms of HSP. However, the different isoforms of HSP changed in parallel in the present study. Mechanisms underlying the discrepancy between the studies remain to be further explored.

In summary, the expression of HSP70, HSP32 and HSP25 was increased in the clipped kidney at both weeks 1 and 4 in 2K1C hypertension. On the contrary, the contralateral kidney of 2K1C rats and the remnant kidney of DOCA-salt rats showed a delayed increase: the expression was not significantly altered at week 1, but increased at week 4. It is suggested that the expression of HSP in the kidney may be differentially regulated according to the duration as well as the model of hypertension.

ACKNOWLEDGEMENTS

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