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European Journal of Pharmacology 487 (2004) 199–203
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Expression of heat shock protein 70 and its mRNAs during ischemia–reperfusion in the rat prostate

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Received 20 October 2003; received in revised form 10 December 2003; accepted 21 January 2004

Abstract

We investigated the expression of heat shock protein (HSP) 70 and its mRNAs during ischemia–reperfusion in the rat prostate. Eight-week-old rats were divided into six groups: a control group, a 30-min ischemia group, and 30-min ischemia + 30-min, 60-min, 1-day, and 1-week reperfusion groups (groups A, B, C, D, E, and F, respectively). In vivo real-time blood flow and HSP 70-1 and 70-2 mRNAs and proteins in the prostate were measured using laser Doppler flow meter, real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) methods, respectively. Clamping of the aorta decreased blood flow to 10% of the basal level. The expressions of HSP 70-1/2 mRNAs increased in groups B, C, and D, and decreased in groups E and F. The expression of HSP 70 proteins was increased after a short interval of increase in their mRNAs. Our data indicated that the expressions of HSP 70 proteins and their mRNAs are dramatically changed during ischemia–reperfusion in the rat prostate.

Keywords: Prostate; Ischemia–reperfusion injury; Heat shock protein 70; Real-time PCR; (Rat)

1. Introduction

Although benign prostate hyperplasia is common in elderly males, its etiology and clinical symptoms are not fully understood (MacConnell, 1998). The clinical manifestations of benign prostate hyperplasia are thought to be primarily due to bladder neck obstruction, which may be caused by compression of the prostatic urethra (Lepor and Machi, 1993; MacConnell, 1998). However, the mechanisms of bladder neck obstruction remain unclear. Moreover, studies have shown a poor correlation between clinical symptoms of the lower urinary tract and either prostate volume or histological prevalence of prostate hyperplasia (Dorflinger et al., 1988; Bosh et al., 1995). Recently, a correlation was demonstrated among prostatic fibrosis, urinary flow impairment and increased postvoid residual urine (Bercovich et al., 1999). Some studies have revealed that the prostate depends on androgens for its structural and functional integrity. Adrenergic steroids regulate prostate size by repressing prostate cell apoptosis and stimulating prostate cell proliferation (Peters and Walsh, 1987). Shabsigh et al. (1998) demonstrated that castration results in a rapid and significant reduction of blood flow to the mature rat ventral prostate gland and an increase in the apoptosis of epithelial cells. In a related study, transient ischemia was shown to induce apoptosis in the rat ventral prostate (Lekas et al., 1999). Moreover, prostatic infarction has been reported to be significantly increased in patients with acute urinary retention (Strrachan et al., 1993). These reports suggest that blood flow and ischemia–reperfusion play an important role in the normal functioning of the prostate. Heat shock proteins (HSP) are induced by many physiological stressors and are thought to be critical in conferring this protection. Both heat stress and ischemia–reperfusion cause extensive cytoskeletal and mitochondrial damages and uncoupling of oxidative phosphorylation. Heat shock proteins, a diverse family of inducible and constitutive stress proteins, are thought to limit injury and accelerate recovery by refolding disrupt red proteins and preventing deleterious peptide interaction (Hunt and Morimoto, 1985; Leung et al., 1990). In mammalian cells, the inducible 70-kDa heat shock protein (HSP 70) is the most abundant heat shock protein and most closely linked to cytoprotection from a variety of dangerous events such as thermal injury or ischemia–reperfusion injury (Hunt and Morimoto, 1985;

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doi:10.1016/j.ejphar.2004.01.021

Leung et al., 1990). In both rats and humans, heat shock protein 70 is encoded by two genes that differ only in the 3′-untranslated region. This similarity between these genes, HSP 70-1 and HSP 70-2, which encode identical proteins, is exceptional. The fact that two genes encode identical proteins may be due to a need to express the same protein under different circumstances (Walter et al., 1994; Akcetin et al., 1999). The present study was undertaken to investigate the effect of ischemia–reperfusion on the biochemical and pharmacological characteristics of heat shock protein 70-1 and 70-2 mRNAs, and heat shock protein 70 protein levels in the rat prostate.

2. Materials and methods

2.1. Production of the animal model

All animal experiments were performed in accordance with the guidelines set by the Tottori University Committee for Animal Experimentation. In the genetic and biochemical studies, 8-week-old male Wistar rats were divided into six groups: a control group, a 30-min ischemia group, and 30-min ischemia + 30-min, 60-min, 1-day, or 1-week reperfusion groups (groups A, B, C, D, E, and F, respectively). The prostates were subjected to ischemia–reperfusion by means of a previously described method with a minor modification (Saito et al., 1998; Saito and Miyagawa, 1999). Under pentobarbital anesthesia (50 mg/kg, ip), the abdominal aorta was clamped just above its bifurcation using a small clip (Sugita standard aneurysm clip; holding force 145 g; Mizuho Ikakogyo, Tokyo) for 30 min. Reperfusion of the prostate was accomplished by removing the clip.

2.2. Measurement of blood flow in the prostate

Blood flow in the ventral rat prostate was measured with a laser Doppler flow meter (BRL-100; Bioresearch, Nagoya, Japan) as previously reported, with a minor modification (Saito and Miyagawa, 1999, 2001). Briefly, under pentobarbital anesthesia, the probe was attached to the ventral prostate and used to measure blood flow before, during, and after ischemia (30 min ischemia followed by 30 or 60 min reperfusion). Blood flow data were expressed as a percentage of the basal level.

2.3. Tissue preparation

For genetic studies, the rest of the prostate was chopped, frozen in liquid nitrogen, and stored at −80 °C until used. Heat shock protein 70-1/2 mRNAs in the experimental bladder were measured by real-time polymerase chain reaction (PCR) methods.

2.4. Real-time PCR (quantification of heat shock protein 70 mRNAs)

The RNA was purified by RNeasy Mini Kit (Quiagen, Valencia, CA) according to the manufacturer's instructions. The reverse transcriptase (RT) mixture (30 μl) containing 2 ml of total RNA was made and incubated at 37 °C for 60 min by a previously reported method (Ueta et al., 2003). One milliliter of the mixture was used for real-time PCR. Real-time PCR was carried out using a LightCycler thermal cycler system with a Light Cycler SYBR Green I kit according to the manufacturer's instructions (Roche Diagnostics, Tokyo, Japan) (Wittwer et al., 1997). The following primers were used: 5′-TTTCTGGCTCTCAGGGTGTT-3′(forward) and 5′-CTGTACACAGGGTGGCAGTG-3′ (reverse) for heat shock protein 70-1, and 5′-GCTACAAGGCGGAGGACG-3′ (forward) and 5′-AGATCACACCTGGAGCGCC-3′ (reverse) for heat shock protein 70-2. A total of 10 ml of solution was used for the sample. The PCR products of heat shock protein 70-1 and heat shock protein 70-2 were subcloned into pGEM-T vectors, and the plasmids were amplified, purified, and diluted for the standard curve. The specificity of the reaction was confirmed by melting curve analysis and 2% agarose gel electrophoresis. The following primers for the beta actin gene were used as the internal standard and analyzed by real-time PCR using the same RT mixture: 5′-CCTCTATGCCAACACAGT-3′ and 5′ -AGCCACCAATCCACACAG-3′ .

2.5. Measurement of heat shock protein 70 in the prostate

For biochemical studies, the rest of the bladder was chopped, frozen in liquid nitrogen, and stored at −80 °C until used. The expression of heat shock protein 70 was measured using a StressXpress HSP 70 enzyme-linked immunosorbent assay (ELISA) kit (Stressgen Biotechnologies, Victoria, BC, Canada) according to the manufacturer's instructions.

2.6. Data analysis

Data on blood flow in the prostate were expressed as a percentage of the control levels during ischemia–reperfusion. The expressions of heat shock protein 70-1/2 mRNAs were quantified according to the expression of beta actin mRNAs in the experimental rat prostate. The expressions of heat shock protein 70-1/2 mRNAs in the prostate were compared to those of the controls using the following ratio:

$$\text{Ratio of expression of heat shock protein 70-1/-2 mRNA (Group B} - \text{F)} = \frac{\text{Heat shock protein 70-1/-2 mRNA (Group B} - \text{F)/beta actin mRNA (Group B} - \text{F)}}{\text{Heat shock protein 70-1/-2 mRNA (Group A)/beta actin mRNA (Group A)}}$$

The expression of heat shock protein 70 was quantified according to comparison of the protein concentrations. Statistical comparison of differences between groups was performed using analysis of variance and Fisher's multiple comparison test. Values of $p < 0.05$ were considered to indicate statistical significance.

2.7. Drugs and chemicals

A kit for colorimetric assay of HSP 70 was purchased from Stressgen Biotechnologies (StressXpress HSP 70 ELISA kit). All other chemicals were available commercially and of reagent grade.

3. Results

3.1. Measurement of blood flow in the prostate

Clamping of the rat abdominal aorta decreased blood flow in the rat ventral prostate to 5–15% of the basal level measured before clamping (Table 1). After removal of the clip, the blood flow recovered to the basal level within 5 min, and the recovered level was maintained for 60 min.

3.2. Measurement of heat shock protein mRNAs in the prostate

Table 2 shows the expression of heat shock protein 70-1/-2 mRNAs in the experimental rat prostates. The expression of heat shock protein 70-1 mRNAs was increased after the ischemia (4.33-fold) and was highest in the group receiving 30 min of ischemia and 30 min of reperfusion (8.69-fold). After 30 min of ischemia and 60 min of reperfusion, the expression of heat shock protein 70-1 mRNAs in the prostate returned to the level observed after 30 min of ischemia. The expression of heat shock protein 70-1 mRNAs after 1 day or 1 week of reperfusion was similar to the level in the controls. The expression of heat shock protein 70-2 mRNAs showed the same trend as the expression of heat shock protein 70-1 mRNAs during ischemia–reperfusion.

Table 1
Blood flow in the prostate during ischemia-reperfusion

Base	Ischemia	Reperfusion	
		30 min	60 min
100 ± 0	11.5 ± 2.5*	110.3 ± 11.5	98.6 ± 15.6

Rat prostates were subjected to the ischemia (30 min) and reperfusion (60 min) by clamping of the abdominal aorta. Data of blood flow were expressed % of control levels. Data are mean ± S.E.M. of 6–7 separate determinations in each group.

* Significantly different from basal and reperfusion (30 and 60 min) levels ($p < 0.05$ is level of significance).

Table 2
Expression of heat shock protein 70-1 and heat shock protein 70-2 mRNAs in the prostate

Groups	HSP 70-1	HSP 70-2
A	1.00 ± 0	1.00 ± 0
B	4.33 ± 0.70*	1.23 ± 0.20
C	8.69 ± 1.54**	2.12 ± 0.30***
D	3.96 ± 1.80*	1.80 ± 0.14***
E	1.46 ± 0.24	0.93 ± 0.23
F	1.51 ± 0.33	1.12 ± 0.32

Prostates were prepared from rats immediately after the ischemia (30 min) (B), ischemia-reperfusion (30 and 30 min) (C), ischemia-reperfusion (30 and 60 min) (C), ischemia-reperfusion (30 min and 1 day) (E), ischemia-reperfusion (30 min and 1 week) (F) and controls (A). The expression of HSP 70-1/-2 mRNAs in the prostate were compared to these of each controls. Data are mean ± S.E.M. of 5–7 separate determinations in each group.

* Significantly different from A, C, E and F groups ($p < 0.05$ is the level of significance).

** Significantly different from the other groups ($p < 0.05$ is the level of significance).

*** Significantly different from A, B, E, and F groups ($p < 0.05$ is the level of significance).

3.3. Measurement of heat shock protein 70 in the prostate

Table 3 shows the expression of heat shock protein 70 in the prostate during ischemia–reperfusion. The level of heat shock protein 70 in the control prostates was 25.3 mg/g protein. The level of heat shock protein 70 after 30 min of ischemia (25.5 mg/g protein) was similar to that in the controls. After 30 min of ischemia and 30 min of reperfusion, the level of heat shock protein 70 in the prostate was significantly increased (40.3 mg/g protein), and was maintained at a similar level after 30 min of ischemia and 60 min of reperfusion (42.3 mg/g protein). The expression of heat shock protein 70 after 1 day or 1 week of reperfusion was almost identical to the level in the controls (28.0 and 22.5 mg/g protein, respectively).

Table 3
Expression of heat shock protein 70 in the prostate

Groups	HSP 70 (mg/g protein)
A	25.3 ± 2.3
B	25.5 ± 0.5
C	40.3 ± 0.7*
D	42.3 ± 0.3*
E	33.0 ± 0.9
F	22.5 ± 0.7

Prostates were prepared from rats immediately after the ischemia (30 min) (B), ischemia-reperfusion (30 and 30 min) (C), ischemia-reperfusion (30 and 60 min) (C), ischemia-reperfusion (30 min and 1 day) (E), ischemia-reperfusion (30 min and 1 week) (F) and controls (A). Data are mean ± S.E.M. of 5–7 separate determinations in each group.

* Significantly different from A, B, E and F groups ($p < 0.05$ is the level of significance).

4. Discussion

In this study, we demonstrated the expression of heat shock protein 70 and its mRNAs during ischemia–reperfusion in the rat prostate. Clamping of the aorta decreased blood flow to approximately 5–15% of the basal level measured before the clamping. In the control group, expressions of heat shock protein 70-1/2 mRNAs were detected. Expressions of heat shock protein 70-1 and 2 mRNAs increased in groups B, C, and D, and decreased in groups E and F. The expression of heat shock protein 70 was increased after a short interval of the expression of their mRNAs (groups C and D). Both the heat shock protein mRNAs and proteins were normalized after 1 day of reperfusion. Our data indicated that heat shock protein 70 mRNAs were increased quickly and were decreased in a relatively early phase of reperfusion. The protein levels of heat shock protein 70 were increased after a short interval of increase in their mRNAs.

The prostate gland is an androgen-dependent organ that requires testosterone for normal growth, development, differentiation, and function (Peters and Walsh, 1987). Ischemia in the prostate is observed after castration (Shabsigh et al., 1998). Recent studies have indicated the regulation of vascular endothelial growth factor expression and decrease blood flow through the rat ventral prostate after castration (Shabsigh et al., 1998). Castration reduces the total volume of blood vessels and endothelial cell in the rat prostate. The decrease in blood flow has been shown to precede morphological changes in prostatic epithelial cells (Lekas et al., 1999).

In 1970, Mostofi suggested the possible involvement of atherosclerosis in the etiology of benign prostate hyperplasia, because of the high prevalence of vascular risk factors in the elderly male population. Spiro et al. (1974) reported that 85% of their patients had prostatic infarcts with acute urinary retention and that only 3% had prostatic infarction without acute urinary retention. These reports suggest that prostatic infarction is often observed clinically, and that ischemia and subsequent reperfusion may play an important role in the pathology of the prostate. Kozlowski et al. (2001) reported that chronic prostate ischemia leads to stromal fibrosis, glandular cystic atrophy, and impaired smooth muscle relaxation in the rabbit. Kazem et al. (2003) recently reported that chronic ischemia alters prostate structure and increases prostatic smooth muscle contraction. These data suggest that ischemia and subsequent reperfusion induce structural and functional alterations including fibrosis in the prostate. On the other hand, Park et al. (1998) reported that prostatic atrophy can be induced by infarction in rats and that prostatic infarction has potential as a new therapeutic strategy for the treatment of benign prostate hyperplasia. Lekas et al. (1999) reported that transient and relative ischemia induce apoptosis in the glandular epithelium in the ventral prostate of rats.

In the present study, clamping of the aorta decreased blood flow to 5–15% of the basal level measured before clamping. After removal of the clip, the blood flow immediately returned to the basal level. It is well known that the laser Doppler method does not measure blood flow per se, but rather the movement of the red blood cells. Nonetheless, this movement of red blood cells has been well correlated to blood flow, and thus is generally used for blood flow measurement. The movement of blood flow during ischemia–reperfusion in the prostate is very similar to that in the bladder, and our data were in agreement with the previous report (Lekas et al., 1999; Saito and Miyagawa, 1999). In the current manuscript, clamping the aorta induces only partial ischemia. Technically, it is very difficult to induce total ischemia in the prostate because of collateral circulation. There is a possibility that total ischemia may induce less reperfusion injury in the prostate.

In mammalian cells, heat shock protein 70 is the most abundant heat shock protein and most closely linked to cytoprotection from a variety of dangerous events, such as thermal injury or ischemia–reperfusion injury (Hunt and Morimoto, 1985; Leung et al., 1990). There are increasing evidences that ischemia–reperfusion induces heat shock protein 70 in some organs, e.g., the kidney and heart (Akcetin et al., 1999; Bidmon et al., 2000; Hutter et al., 1994; Kelly et al., 2001). Heat shock proteins are thought to limit injury and accelerate recovery by refolding disrupt red proteins and preventing deleterious peptide interaction. In the kidney, it has been shown that there is a significant induction of both heat shock protein 70-1/-2 genes immediately after ischemia. While heat shock protein 70-1 mRNA expression constantly increased during reperfusion, heat shock protein 70-2 mRNA was strongly induced (threefold) during reperfusion only after brief periods (10 min) of ischemia in the kidney (Akcetin et al., 1999; Bidmon et al., 2000; Hutter et al., 1994; Kelly et al., 2001). These data suggest that heat shock protein 70-1/-2 mRNAs are induced immediately after the insult. In this study, heat shock protein 70-1/-2 mRNAs were increased immediately after induction of ischemia. From the data presented in study, it is difficult to distinguish between induction of gene expression and degradation of HSP 70 mRNA. In this regard, our data are in agreement with previous reports on other types of tissue (Akcetin et al., 1999; Bidmon et al., 2000; Hutter et al., 1994; Kelly et al., 2001). Many reports indicate that when HSP 70-1/2 mRNA levels are increased, there is not induction of ischemia but induction of reperfusion (Akcetin et al., 1999; Bidmon et al., 2000; Hutter et al., 1994; Kelly et al., 2001). However, in this experiment, ischemia for 30 min induced an increase of HSP 70-1 mRNA. This may be due to clamping of aorta resulted in partial ischemia (approximately 10% of basal level) of the prostate and not complete ischemia of the organ. Interestingly, protein levels of heat shock protein 70 were increased after 30 min of ischemia and 30 min of reperfusion in this study. Our data suggest that the expres-

sion of heat shock protein 70 proteins is increased after a short interval of the expression of their mRNAs in the prostate, and that this increased expression continues longer than that of mRNA in the prostate.

In the heart and other organs, ischemia–reperfusion injury has been reported to induce fibrosis and dysfunction. The patho–physiological role of ischemia–reperfusion in the prostate, however, remains unclear and warrants further study.

Acknowledgements

This study was supported by a Grant from the Ministry of Education, Science, and Culture of Japan (#14704041).

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