Restriction Fragment Length Polymorphism of hsp70 Gene, Localized in the RT1 Complex, Is Associated With Hypertension in Spontaneously Hypertensive Rats

Pavel Hamet, Dewen Kong, Michal Pravenec, Jaroslav Kunes, Vladimir Kren, Pavel Klir, Yu-Lin Sun, and Johanne Tremblay

Previous studies from our laboratory have demonstrated that the intermediate phenotype of thermosensitivity is present in hypertensive mice and rats. Increased expression of hsp70 caused by increased transcription rate was demonstrated in vivo, in organs, and in cultured cells from spontaneously hypertensive rats and hypertensive mice. In this study, a polymorphism of this gene was revealed with BamHI enzyme by using a human hsp70 probe. A 4.4-kb fragment was visualized in normotensive rats (Brown-Norway BN.Ix and Sprague-Dawley), and a 3.0-kb fragment was found in spontaneously hypertensive rats (SHR) of three different origins and in Wistar and Buffalo rats. Both fragments were present in the Wistar-Kyoto rat strain. The present study mapped the polymorphism of hsp70 into the RT1 complex in BN.1K and SHR.1N congenic strains. The hsp70 restriction fragment length polymorphism is associated with a blood pressure difference of 15 mm Hg in recombinant inbred strains. These results justify the search for a mechanism by which hsp70 could influence blood pressure. (Hypertension 1992;19:611-614)

Keywords • restriction fragment length polymorphisms • heat-shock proteins • DNA, recombinant • spontaneously hypertensive rats

Highened thermosensitivity potentially constitutes a distinct intermediate phenotype of hypertension. Increased environmental heat susceptibility has been demonstrated in hypertensive rats and mice.1-6 Our prior studies have suggested from observation of F2 and backcross mice populations that this abnormal thermosensitivity is determined by a specific locus cosegregating with blood pressure (Tms).7 Environmental temperature has an impact on the expression of hypertension in animals and humans.8-9 Data from our laboratory suggest that increased thermosensitivity persists even in vitro, i.e., in cultured cells from vascular smooth muscle and cardiomyocytes, and may be genetically determined. The best candidate genes for this thermosensitivity are heat stress genes. This group of genes, classified by the molecular weight of their protein products, is expressed in a variety of cells of all organisms after application of stimuli, including heat, mechanical stress, a-adrenergic agonists, and calcium ionophore. A major representative of this group of genes is hsp70 (for review, see Reference 10). Using mouse hsp68 and human hsp70 probes from the hsp70 family of genes, we have established that there is an increased transcription rate as well as messenger RNA (mRNA) accumulation of a major representative of hsp70.11,12 However, preliminary studies indicate that the mRNA accumulation is only transient and that the protein product becomes actually even less abundant with time in hypertensive animals after stress exposure of whole animals, isolated organs, and cultured cells.13-15 At least two copies of hsp70 were localized in the major histocompatibility complex of humans,16 mice,17 and rats.18 The area in which hsp70 is localized is of interest since it contains other genes potentially relevant to environmental interactions, such as 21-hydroxylase (21-OH) and TNFa. To evaluate whether hsp70, which is increasingly expressed in hypertensive cells, segregates with blood pressure, we have searched for polymorphisms in various hypertensive and normotensive rat strains and studied segregation with hypertension in recombinant inbred (RI) and congenic strains.

Methods

Animals

Spontaneously hypertensive rats (SHR) of three different origins were used in the present study. Two of
them were purchased from commercial North American sources (Taconic Farms, Inc., Germantown, N.Y., and Charles River, Wilmington, Mass.), and the third was obtained from the Czechoslovak Academy of Sciences, Prague (but originated from OLAC, England). The normotensive animals were Brown-Norway BN.1x progenitors (hereafter referred to as BN) of RI strains and Wistar-Kyoto (WKY) rats from Taconic Farms. The RI strains were partly from the Czechoslovak Academy of Sciences and partly from the Biology Department, Faculty of Medicine, Charles University, as previously reported,19 representing a set of 30 fully inbred homozygous strains. The congenic strains were BN.1x.1K with the RT1<sup>b</sup> haplotype on a normotensive background and SHR.1N with the RT1<sup>h</sup> haplotype on a hypertensive background.19 Normotensive strains of Wistar, Buffalo, and Sprague-Dawley rats were also used.

**DNA Preparation, Electrohoresis, and Southern Hybridization**

High molecular weight genomic DNA was processed from adult livers of different rat strains as described by Sambrook et al.20 After restriction enzyme digestion, DNA was electrophoresed on 0.8% agarose gel for 42 hours at 25 V and then transferred onto a Nyl<sup>®</sup> membrane (Amersham Canada Ltd., Oakville, Canada) by using Vacugen (Pharmacia-LKB, Baie D'Urfe, Canada). The hybridization process has been described previously.21 Autoradiography was performed with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.).

**DNA Probes**

To prepare human hsp70 probes, HindIII–Sph I insert (2.78 kb) and 5′–Pst I fragment (1.2 kb) in PHHSP70 plasmid, kindly donated by Dr. R.I. Morimoto, were processed by elution from agarose gel after restriction enzyme digestion and electrophoresis.21 These DNA fragments were labeled with [α<sup>32P</sup>]dCTP and DNA polymerase (Klenow fragment) by random-primer extension.22 Mouse hsp68–containing plasmid was provided by Dr. L.A. Moran.23 The probe was prepared as described elsewhere.11

**Results**

Digestion by several restriction enzymes, such as Pst I, Xba I, Xho I, and Sac I, did not reveal any polymorphism of hsp70. Only BamHI digestion allowed the visualization of a clear polymorphism between normotensive and hypertensive animals. This enzyme also demonstrated 15-kb and 8-kb fragments identical in all animals, but a distinct 4.4-kb fragment was present only in BN and WKY strains. A shorter fragment of 3 kb, although also evident in WKY rats, was seen in all SHR strains of Prague, Taconic, and Charles River origin. Noticeably, in WKY rats, both bands were visible but the intensity of hybridization was less as compared with that of BN and SHR for both fragments. An example of this polymorphism is illustrated in Figure 1. Both human hsp70 probes (2.78 kb and 1.2 kb) and the mouse hsp68 probe gave the same results. These data demonstrate the homogeneity of this gene polymorphism in hypertensive strains of various origins and the presence of larger fragments in both normotensive strains. Additional experiments with other normotensive strains have revealed the presence of the 3.0-kb fragment in Buffalo and Wistar rats, and the 4.4-kb fragment was found in BN and Sprague-Dawley rats. The different hsp70 alleles characterized by hsp70 polymorphism are summarized in Table 1.

Use of the RT1 congenic strains SHR.1N and BN.1K enabled us to map this polymorphism to the rat major histocompatibility complex (RT1) on chromosome 20 where the RT1 complex is now assigned to linkage group IX.24 The mean blood pressure of the Prague SHR strain was 181±4 mm Hg, while that of the SHR.1N congenic strain was significantly (p<0.001) lower (156±4 mm Hg). It is evident from Figure 2 that the congenic strains with an RT1<sup>b</sup> haplotype, whether on a hypertensive (SHR.1N) or normotensive (BN.1x) background, demonstrated 15-kb and 8-kb fragments identical in all animals, but a distinct 4.4-kb fragment was present only in BN and WKY strains. A shorter fragment of 3 kb, although also evident in WKY rats, was seen in all SHR strains of Prague, Taconic, and Charles River origin. Noticeably, in WKY rats, both bands were visible but the intensity of hybridization was less as compared with that of BN and SHR for both fragments. An example of this polymorphism is illustrated in Figure 1. Both human hsp70 probes (2.78 kb and 1.2 kb) and the mouse hsp68 probe gave the same results. These data demonstrate the homogeneity of this gene polymorphism in hypertensive strains of various origins and the presence of larger fragments in both normotensive strains. Additional experiments with other normotensive strains have revealed the presence of the 3.0-kb fragment in Buffalo and Wistar rats, and the 4.4-kb fragment was found in BN and Sprague-Dawley rats. The different hsp70 alleles characterized by hsp70 polymorphism are summarized in Table 1.

**Rat strain**

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>hsp70 alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wistar</td>
<td>3.0 kb</td>
</tr>
<tr>
<td>SHR (Prague)</td>
<td>--</td>
</tr>
<tr>
<td>SHR (Charles River)</td>
<td>+</td>
</tr>
<tr>
<td>SHR (Taconic)</td>
<td>--</td>
</tr>
<tr>
<td>BUF</td>
<td>+</td>
</tr>
<tr>
<td>BN</td>
<td>--</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>--</td>
</tr>
<tr>
<td>WKY</td>
<td>+</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; BUF, Buffalo normotensive rats; BN, Brown-Norway normotensive rats; WKY, Wistar-Kyoto rats.

**Table 1. Alleles Characterized by hsp70 Polymorphism**

**Figure 1. Autoradiogram showing restriction fragment length polymorphism between normotensive Wistar-Kyoto (WKY), Brown-Norway (BN), and spontaneously hypertensive rat (SHR) strains.**
background, possess the 4.4-kb fragment, while those with the RT1\(^k\) haplotype on SHR or normotensive backgrounds (BN.1K) have the 3.0-kb fragment. This was confirmed in RI strains.

Figure 3 illustrates a complete association between the RT1\(^k\) haplotype and the 3.0-kb fragment, and the 4.4-kb fragment was observed in all RI strains with the RT1\(^n\) haplotype, demonstrating the absence of intragenic recombination in these strains. Mean blood pressure in the RI strains with the 4.4-kb fragment was 128±3 mm Hg, and in the RI strains with the 3.0-kb fragment it was 143±6 mm Hg. This difference of 15 mm Hg was statistically significant (\(p<0.02\)) and corresponded with data reported previously for RT1\(^n\) and RT1\(^k\) haplotypes.\(^{19}\)

**Discussion**

A polymorphism of \(hsp70\) was demonstrated between normotensive and hypertensive rats. Although \(hsp70\) constitutes a family of genes, the probe used in the present studies revealed that a major representative of this family maps to the RT1 complex. This is unequivocally demonstrated by our results in RI and congenic strains of BN and SHR progenitors and is concordant with the findings in other strains by Wurst and others.\(^{18,25,26}\) These data underline the relevance of the rat RT1 complex as one of the components determining the expression of hypertension and are compatible with the possibility that the genomic abnormality is in the area where \(hsp70\) is located.

It is clear that although the 3.0-kb allele, localized in the RT1\(^k\) haplotype, is present in all SHR tested, it also occurs in the Wistar and Buffalo strains. On the other hand, it should be borne in mind that the RT1 complex contributes by only 15 mm Hg to the expression of hypertension. Our current hypothesis is that the locus for environmental susceptibility lies within the RT1 complex potentially associated with \(hsp70\). Heat susceptibility and \(hsp70\) expression will have to be tested in other strains possessing the 3.0-kb fragment. Interestingly, it has recently been demonstrated that a strain with susceptibility to stress may be selected from the Wistar background.\(^{27}\)

In conclusion, the polymorphism of the \(hsp70\) gene, a member of the \(hsp70\) family that has previously been found to be excessively expressed in hypertension, maps to the RT1 complex and is thus associated with a 15 mm Hg increment of blood pressure.

**Acknowledgments**

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tions, and Louise Chevrefils for preparing and Ovid Da Silva for editing this manuscript.

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