Thermosensitivity, A Possible New Locus Involved In Genetic Hypertension

Danielle Malo, Gunther Schlager, Johanne Tremblay, and Pavel Hamet

Spontaneously hypertensive mice have been characterized as more sensitive to environmental heat than normotensive mice. A breeding program was therefore initiated to examine the possible genetic link between thermosensitivity and hypertension. Crossbreeding of spontaneously hypertensive mice with randomly bred normotensive mice produced F₁ hybrids, which were then intercrossed to create a F₂ population. Thermosensitivity was measured with a noninvasive method. The rate of body temperature increase was significantly (p<0.001) higher in the hypertensive mice (1.74±0.04°C/min) compared with normal controls (1.13±0.03°C/min). The frequency distribution of the rate of body temperature increase among the progenies was consistent with the hypothesis that a single gene locus determines the observed difference in thermosensitivity between normal and hypertensive mice. The allele that determines the rate of body temperature increase in normal mice was dominant in relation to the allele contributed by hypertensive mice. In the F₂ population, a bimodal distribution determined two phenotypes: less than 1.40°C/min and greater than 1.40°C/min. A significant difference (p<0.01) in blood pressure of 11 mm Hg was observed between these two phenotypes. In addition, a positive correlation (p<0.01) was noted between the rate of body temperature increase and blood pressure in the F₃ progeny. We conclude that there is possibly a single locus controlling thermosensitivity, which exhibits additive-dominance inheritance. Alleles of this particular trait segregate in part with an increment in blood pressure. (Hypertension 1989;14:121-128)

Essential hypertension has a significant genetic determinant involving several genes.¹ Many genetically hypertensive models have been developed. The main representative is the spontaneously hypertensive rat (SHR),² a model that has contributed significantly to our knowledge, but that is deficient in several aspects, especially in the lack of suitable controls.³,⁴ Spontaneously hypertensive mice (SHM) have been developed by eight-way crossbreeding of unrelated inbred mouse strains, followed by random mating with a randomly bred normotensive line used as controls.⁵ By the 24th generation, the divergence between SHM and low blood pressure mice is about 60 mm Hg (142±3 mm Hg vs. 81±2 mm Hg).⁶ This 60 mm Hg difference is due to 8–20 loci.⁷ The blood pressure value is 102±3 mm Hg for the randomly bred normotensive line.⁸ There is no difference in pulse rate between the three lines.⁹ Body weight is significantly higher in the randomly bred line compared with both low blood pressure mice and SHM.⁹ We have compared some characteristics of this animal line with the widely used SHR strain. Decreased longevity,¹⁰ neonatal cardiac hyperplasia,⁴,¹¹ lower brain norepinephrine levels,¹² and the presence of a calmodulin activator in the heart and kidney¹³ appear to be common determinants in SHR and SHM.

Another common characteristic of SHR and SHM is their increased sensitivity to environmental temperature.⁵,⁷,¹⁴–¹⁹ Greater heat sensitivity does not appear to occur only in vivo but persists at the cellular level in cardiomyocytes obtained from neonatal SHR. The cellular death rate at 45°C is significantly higher in SHR than in Wistar-Kyoto (WKY) control rats.⁴ The production of hybrids from selected strains can be used to define which biochemical or physiological differences between normotensive and hypertensive lines are either genetically linked or coincidental.¹ The two selected lines showing

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extreme values for that trait are crossed to produce $F_1$ hybrid. Subsequently, the $F_1$ progeny are intercrossed to create $F_2$ population and are crossed back to the two parental lines. These genetic crossings allow the identification of traits that either cosegregate or are independent of genes responsible for high blood pressure. If the trait is determined by genes influencing blood pressure, then it will remain associated with an increment of blood pressure in genetically segregated populations, and if the same genes affect two different traits, their expression should be correlated in the segregated $F_2$ population.15

This method has been applied to study the relation between many traits and blood pressure in rats: an association has been found between blood pressure and the steroid profile of 180H-deoxycorticosterone in Dahl salt-resistant and salt-sensitive rats,20 renal esterase isoenzymes in SHR and WKY rats,20 vascular responsiveness to cobalt in SHR and Dahl salt-resistant rats21 and to norepinephrine in SHR stroke-prone (SHRSP) and WKY rats,23 red blood cell membrane Na"+K" cotransport in Milan hypertensive and normotensive strains24 and in SHR and WKY rats,25 and renin heterogeneity and potassium efflux across lymphocyte membranes in SHRSP and WKY rats.26 Conversely, a dissociation of traits and blood pressure has been observed for genetic hyperactivity and sodium balance in SHR and WKY rats.26,27 One study using this method in mice was unable to demonstrate an association with blood pressure for hyperactivity.30

The present investigation was designed to apply this genetic method to examine the relation between thermosensitivity and hypertension. Since we have demonstrated that heat treatment attenuates the expression of genetic hypertension,4,31 we measured blood pressure and the rate of body temperature increase in normal mice, SHM, and their hybrids.

**Materials and Methods**

**Breeding**

The original colony of SHM was developed in 1974 via a selection program from an eight-way cross of unrelated inbred strains of mice.5 SHM from this original strain and their age-matched random controls were bred at the Clinical Research Institute of Montreal. The mice were maintained at an ambient temperature of 22±1°C with a 12-hour light/dark schedule throughout the year.

Five males and five females from each line were randomly selected from our colonies. At that time, the SHM were in their 37th generation of selection for blood pressure, which should have fixed the genes in the high blood pressure line. Males and females from each line were mated at 16 weeks of age, after blood pressure and thermosensitivity determinations, to produce $F_1$ hybrids of SHM and normal mice. From this crossbreeding, 12 males and 12 females were chosen at random and mated at 16 weeks to obtain a $F_2$ population. Ten $F_1$ males and 10 $F_1$ females were crossed back either to normal mice ($F_1$×normal) or to SHM ($F_1$×SHM) to produce backcross-normal and backcross-SHM populations, respectively.

**Blood Pressure**

Systolic blood pressure was determined in conscious, warmed, and restrained mice at 14 weeks of age by a tail-cuff plethysmographic method, using a programmed electrosphygmomanometer (Narco Biosystems, Houston, Texas) coupled to a unigraph (Gilson Medical Elec., Inc., Middleton, Wisconsin).32 The animals were first restricted for 1 minute on a small warming plate (37°C) in a chamber designed for mice, and restriction time was increased by 1 minute every day for 4 days before the blood pressure measurement on the 5th day. Each estimation was the average of three recordings taken at 10-second intervals. The validity of this method for measurement of blood pressure in mice has been demonstrated previously.33

**Thermosensitivity**

Thermal resistance, as determined by survival time, was initially studied in 10 normal mice and 10 SHM aged 8 weeks. After administration of anesthesia (0.1 mg/g sodium pentobarbital), each mouse was secured in a 50-ml tube that was drilled to allow free circulation of water. Groups of four mice were then placed in a test tube rack and immersed up to their forelimbs in a water bath (Lauda model RC20, Sybron/Brinkman, Pointe Claire, Quebec, Canada) heated to 44.0±0.1°C. Rectal temperature was measured every minute with a thermoprobe sensor.

To avoid the use of survival time as a measure of thermal sensitivity, a nonlethal method was developed for further studies. Mice aged 15 weeks were heat-exposed in the same way for 7 minutes at 44.0±0.1°C, which assures 100% survival. Rectal temperature was recorded continuously during the immersion with a thermoprobe sensor coupled to a recorder (Recordall 5000, Fisher Scientific, Ottawa, Ontario, Canada). The rate of body temperature increase was evaluated by measurement of the slope of the curve obtained between 1 and 3 minutes of immersion. The mice were then allowed to recover from the anesthesia.

**Statistical Analysis**

Analyses of variance and covariance, with body weight as covariable, compared blood pressure and the rate of body temperature increase between the different populations. Student's $t$ test for grouped data was used to compare blood pressure of the two phenotypes in the $F_2$ progeny. The relation between blood pressure and rate of body temperature increase was determined by standard parametric regression techniques. Mather-Jinks biometrical genetic analysis with the $\chi^2$ goodness of fit was employed to assess the significance of deviation of the observed frequency of the rate of body temperature increase from predic-
TABLE 1. Response to Acute Heat Stress at 44° C in Normal and Spontaneously Hypertensive Mice

<table>
<thead>
<tr>
<th></th>
<th>Normal mice</th>
<th>SHM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating to 43° C (min)</td>
<td>13±1</td>
<td>7±1*</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>39±3</td>
<td>30±2†</td>
</tr>
<tr>
<td>Rate of body temperature</td>
<td>1.1±0.1</td>
<td>1.5±0.1†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHM, spontaneously hypertensive mice. *p<0.001. †p<0.05.

Table 1 depicts the lethal sensitivity of mice to body heating. Body temperature reached 43° C significantly faster in SHM (by 46%). The mean time before death ensued on exposure to 44° C heat was significantly shorter (23%) in SHM than in the normal control mice. Initial body temperature was lower in the SHM, but at 1 minute of immersion, this difference disappeared (Figure 1). Body temperature rose at a quicker rate in the SHM compared with normal mice during the first 3 minutes of heat exposure (Table 1 and Figure 1). After 7 minutes of heat exposure, it was significantly higher in SHM compared with their controls (Figure 1).

To determine the genetic basis of these strain differences, standard genetic crosses were made between normal mice and SHM, and the response to heat stress, measured as the rate of body temperature increase, was studied in these populations. Figure 2 shows the frequency distribution of blood pressure in normal mice, SHM, and their genetic cross populations. Normal mice and SHM displayed extreme blood pressure values. In the F1 population, mean blood pressure was equivalent to the midparental value (114 mm Hg). The backcross mice had values that approached those of their normal or SHM parents. A unimodal distribution of blood pressure was noted in all populations with the exception of the backcross to SHM. In addition, there was a break in F2 distribution.

Figure 3 illustrates the frequency distribution of the rate of body temperature increase in the same six populations. Normal mice and SHM demonstrated extreme mean values for the rate of body temperature increase (1.13±0.04° C/min vs. 1.74±0.04° C/min). In the F1 population, the mean rate of body temperature increase (1.21±0.04° C/min) was displaced toward that of the normal parent. The backcross with the normal population had values that resembled their normal parents, whereas backcross with SHM had values smaller than those of SHM.
Figure 3. Graphs of frequency distribution of rate of body temperature increase in normal mice, spontaneously hypertensive mice (SHM), F\(_1\) (normal x SHM), F\(_2\) (F\(_1\) x F\(_1\)), backcross-normal (F\(_1\) x normal), and backcross-SHM (F\(_1\) x SHM). Rate of body temperature was measured in 15-week-old mice. Numbers of animals on the y-axis are expressed as percentage of total for a given number of mice.

Figure 4. Representation of genetic triangle showing average blood pressure (Panel A) and rate of body temperature increase (Panel B) in segregating and non-segregating generations. SHM, spontaneously hypertensive mice; F\(_1\), normal mice x SHM; F\(_2\), F\(_1\) x F\(_1\); backcross-normal, F\(_1\) x normal mice; backcross-SHM, F\(_1\) x SHM.

Figure 5. Graph of frequency distribution of rate of body temperature increase in normal mice, spontaneously hypertensive mice (SHM), F\(_1\) (normal x SHM), F\(_2\) (F\(_1\) x F\(_1\)), backcross-normal (F\(_1\) x normal), and backcross-SHM (F\(_1\) x SHM). A value of 1.40°C/min for rate of body temperature increase was selected as division point between thermoresistant and thermosusceptible mice. Mice with values higher than 1.40°C/min were considered thermosensitive. The F\(_1\) hybrids derived from the cross of thermoresistant and thermosensitive mice were almost uniformly thermoresistant, implying that thermoresistance was dominant over thermosensitivity. Among the progeny of F\(_1\) hybrids backcrossed to the thermosensitive progenitor, approximately 50% of mice were found to be thermoresistant, whereas the F\(_1\) hybrids showed bimodality. Bimodality was observed in the F\(_2\) progeny. Reciprocal F\(_1\) crosses (normal female x SHM male or normal male x SHM female) did not differ in blood pressure and thermosensitivity.

The results were further analyzed by representation of a genetic triangle (Figure 4). For blood pressure (Figure 4A), the parental means and F\(_1\) values were almost on a straight line, demonstrating additivity of the trait, but the segregating backcross-SHM population was above that line. Epistatic interaction could have accounted for this deviation. Partial dominance of the normotensive parent for the thermosensitivity trait was indicated by a significant displacement of the F\(_1\) value below the midparental value (Figure 4B).

A value of 1.40°C/min of the rate of body temperature increase was selected by inspection as the division point between thermoresistant (normal mice) and thermosensitive mice (SHM) (Figure 5). Mice with values lower than 1.40°C/min were considered thermosensitive, whereas mice with values higher than 1.40°C/min were considered thermoresistant.
hybrids backcrossed to the thermoresistant parents were almost all thermoresistant. Approximately 75% of mice of the F2 generation were thermoresistant. These ratios of thermoresistant-to-thermosensitive individuals of the hybrid and backcross generations are compatible with the hypothesis that the trait of thermosensitivity is under the control of a single gene. Blood pressure was compared with the segregating rate of body temperature increase in the F2 population. Table 2 shows the correlations between blood pressure, rate of body temperature increase, body weight, and heart rate in the F2 progeny. The only significant correlation was between blood pressure and the rate of body temperature increase (r=0.32, p<0.01). In F2 mice, there was a significant blood pressure difference (p<0.05) of 11 mm Hg between the two segregating phenotypes (Table 3). The segregating ratio was 3:1 in this population.

Table 4 enumerates the genetic parameters of blood pressure and the rate of body temperature increase in normal mice, SHM, F1, F2, and backcrosses. The genetic parameters were estimated by the least-squares technique in which six equations, based on the contribution of each family type to m, the midpoint between the selected lines (midparental value); d, deviation of each homozygote from m; h, deviation of each heterozygote from m; SHM, spontaneously hypertensive mice; F1, normal mice × SHM; F2, F1×F1; BC-normal, normal mice × SHM; BC-SHM, SHM × F1.

In fact, the observed F2 value was between the expected midparental value and the expected F1 value for the rate of body temperature increase.

**Discussion**

The conventional approach to demonstrating a genetic link between a trait and blood pressure is to first look for genetic polymorphisms in some biochemical or physiological characters known to influence blood pressure. The relation of these traits to blood pressure is then proven by animal breeding experiments in which the segregation of alleles is studied in the F2 population. The type of inheritance of the character is also determined by this method.1

Heightened heat sensitivity has been noted in many hypertensive animal models, including mice, rats, and pigs,5,7,14,19,35 suggesting that it is linked to hypertension. Our study demonstrates that, during acute heat stress, thermoregulation in SHM is not as efficient as in normal mice. Survival time at 44°C is shorter in SHM compared with normotensive controls, and the rate of the increase in rectal temperature is significantly greater in the former than in the latter. Determination of the rate of body temperature increase was developed as a nonlethal method to evaluate thermal sensitivity. An enhanced temperature increase was developed as a nonlethal method to evaluate thermal sensitivity. An enhanced temperature increase was developed as a nonlethal method to evaluate thermal sensitivity. An enhanced temperature increase was developed as a nonlethal method to evaluate thermal sensitivity. An enhanced temperature increase was developed as a nonlethal method to evaluate thermal sensitivity.

**Table 2. Correlations Between Blood Pressure, Rate of Body Temperature Increase, Heart Rate, and Body Weight in the F2 Population**

<table>
<thead>
<tr>
<th>Correlations</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs. rate of body temperature increase</td>
<td>0.3230</td>
<td>0.0126</td>
</tr>
<tr>
<td>vs. body weight</td>
<td>0.1774</td>
<td>0.1787</td>
</tr>
<tr>
<td>vs. heart rate</td>
<td>-0.1122</td>
<td>0.3975</td>
</tr>
<tr>
<td>Rate of body temperature increase</td>
<td>-0.1359</td>
<td>0.3047</td>
</tr>
<tr>
<td>vs. heart rate</td>
<td>-0.0976</td>
<td>0.4622</td>
</tr>
<tr>
<td>Body weight</td>
<td>-0.0581</td>
<td>0.6619</td>
</tr>
</tbody>
</table>

**Table 3. Blood Pressure of Phenotypes for the Rate of Body Temperature Increase in the F2 Population**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>AA</th>
<th>Aa</th>
<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segregating ratio</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Corresponding phenotype <em>(° C/min)</em></td>
<td>&lt;1.40</td>
<td>&gt;1.40</td>
<td></td>
</tr>
<tr>
<td>Blood pressure <em>(mm Hg)</em></td>
<td>104±2</td>
<td>115±5*</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>43</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

It is impossible to differentiate between AA and Aa genotypes in the F2 population; these are unified in one phenotype (<1.40) in the F2 population. *p<0.05 by Student’s t test for grouped data.
SHM. The F1 value for blood pressure was almost on a straight line between the two parental values, demonstrating additivity of the trait. However, the F2 value was lower than the F1 and the segregating backcross-SHM population was above the line, as shown in the genetic triangle. The cross data for blood pressure did not fit a simple additive-dominance genetic model, but rather looked like a model with epistatic interactions.

It has been demonstrated that the genes affecting blood pressure in mice act additively in crosses between inbred strains with relatively high (BALB/cJ and SWR/5 strains) and low blood pressure (A/J). But crosses between selected lines for high and low blood pressure show some degree of dominance. In the present study, we used the random line developed rather than the low blood pressure line as normotensive controls, which could explain the observation on the mode of inheritance. The random line apparently displays a higher degree of genetic heterogeneity, but it still serves as an adequate control, since this strain underwent simultaneous breeding parallel to the high blood pressure line. The mode of inheritance of blood pressure in our study was similar to that observed in some SHR crosses. Genetic crosses between SHR and three different controls (Wistar-Imamichi, Wistar-Kyoto, and Wistar-Mishima) produced similar results: F1 hybrids showed intermediate blood pressure levels between both parents; the F2 values were always lower than the F1; and the distribution of blood pressure was continuous in each generation. Similar results were obtained by crossing SHR and Dahl salt-resistant rats with the exception of the backcross to SHR in which bimodality was noted.

The mode of inheritance of the rate of body temperature increase appears more simple. The value for F1, which was not significantly different from that of the normal parent, was displaced below the midparental value, and the distribution of the trait in the backcross with the normal parent mimicked that of the normal parent, indicating dominance of the normotensive parents. Frequency distribution in the F2 population was bimodal, showing two phenotypes (<1.400 C/min and >1.400 C/min) and a 3:1 segregation ratio. The ratio of thermoreistant and thermosensitive animals of the different generations are compatible with the hypothesis that the expression of the rate of body temperature increase is controlled by a single dominant gene. As expected, the genetic architecture of the two traits was distinct: thermosensitivity is a trait controlled by a single locus whereas blood pressure is a trait controlled by several loci. The thermosensitivity gene or one closely linked to it would represent one of the loci for hypertension.

To accept that a genetic locus controls a specific trait, as the ones influencing blood pressure, the four criteria put forward by Rapp should be met: 1) a difference in a biochemical or physiological trait between two strains must be demonstrated; 2) the trait must be shown to follow Mendelian inheritance; 3) the genes identified in this way must cosegregate with an increment in blood pressure; and 4) there must be some logical and physiological link between the trait and blood pressure. If these four criteria are satisfied, a strong argument can be made that this trait plays a role in the pathogenesis of hypertension.

The genetic locus controlling the rate of body temperature increase met several of these criteria: 1) the SHM responded to heat stress with a significantly (p<0.001) higher rate of body temperature increase than normotensive mice; 2) in standard genetic crosses between SHM and normal mice, inheritance of the rate of body temperature increase was compatible with a monogenic control; and 3) genes controlling the rate of body temperature increase segregated with an increment in blood pressure in the F2 population; the two phenotypes observed at the locus involved differed in blood pressure by 11 mm Hg. However, blood pressure values were not significantly different between the two phenotypes in the backcross-SHM population. Examples of a similar problem have been presented with other loci, yet the lack of clear correlation both in F2 and backcross-SHM weakens the notion of genetic association of thermosensitivity and hypertension. The lack of full inbreeding or the presence of epistasis, which is in fact underlined by highest blood pressure values in the backcross-SHM population compared with the hypertensive parental line, are possibly responsible for this observation. The third criterion allows estimation of the blood pressure effects of each locus independently of the others. A similar degree of significant differences in blood pressure of 10, 17, and 12 mm Hg was demonstrated between the two phenotypes observed in F2 for vascular responsiveness to cobalt or norepinephrine and renal esterase isoenzyme profile. This type of approach does not allow us to differentiate between pleiotropic effect or close linkage: either the difference in heat sensitivity between normal mice and SHM accounts for the associated difference in blood pressure or that allele closely linked to the thermosensitivity locus causes this blood pressure increment. In our study, we showed that the locus of thermosensitivity or one closely linked could be responsible for 25% of the difference in blood pressure between normal mice and SHM.

The fourth criterion appeared to be fulfilled at least partially. The increased sensitivity to heat, defined as decreased survival time in vivo, was even present in vitro in cardiomyocytes and in aortic smooth muscle cells obtained from SHR, suggesting the primary involvement of heat sensitivity in hypertension. Heat susceptibility may represent an increased sensitivity to environmental effects on blood pressure. Enhanced responses to heat, pain, cold stress, and noise were all demonstrated in experimental and human hypertension. In addition, hypertension may be increased by such stressful stimuli as heavy metal and ethanol. The
appealing possibility that several of these stimuli may involve heat shock genes\textsuperscript{43} deserves further investigation.

Because the locus controlling the rate of body temperature increase in mice meets in principal all four criteria, we suggest that the thermosensitivity locus identified in this study or one closely linked to it potentially represents one of the loci involved in determination of high blood pressure.

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