The objective of this study was to evaluate whether the major histocompatibility complex of the rat can be related to blood pressure (BP) level and BP response to stress. Blood pressure was determined under light ether anesthesia or during moderate restraint stress in normotensive Lewis rats, in rat strains congenic with respect to their RT1 haplotype [LEW.1A (RT1^a)] and LEW.1W (RT1^w)], and in their recombinant lines LEW.1AR1 (RT1^w), LEW.1AR2 (RT1^w), LEW.1WR1 (RT1^a), and LEW.1WR2 (RT1^w). Under light ether anesthesia, systolic blood pressure was similar in Lewis, LEW.1A, and LEW.1W rats. There were also no significant differences in blood pressure in LEW.1AR1 and LEW.1AR2 animals when compared with Lewis or LEW.1A rats. In contrast, BP was significantly increased in LEW.1WR2 rats. On the other hand, moderate restraint stress induced a BP increase in animals of all recombinant lines compared to the respective congenic strains.

These results confirmed our previous finding in recombinant inbred strains about the significant role of RT1 complex in BP regulation. Moreover, our data indicated that BP can be influenced by interaction of individual regions of the RT1 complex on the genetic background of Lewis strain. Am J Hypertens 1996;9:675-680

KEY WORDS: Hypertension, immune system, RT1 complex, stress, congenic strains, major histocompatibility complex, Lewis rat.
three components of the complement system (ie, C2, factor B, and C4)\textsuperscript{11,12} the microsomal cytochrome P-450 steroid 21-hydroxylase,\textsuperscript{13} the cytokines tumor necrosis factor-\(\alpha\) and -\(\beta\),\textsuperscript{14,15} and others. Recently, the gene for major heat shock protein (\textit{hsp70}) was recognized within MHC.\textsuperscript{16,17}

The rat MHC (RT1 complex) is located on chromosome 20\textsuperscript{18} and encompasses a chromosomal region of about 2 to 4 cm according to recombination data. The Glo-1r and Acry-1 genes are located in the neighborhood of the RT1 complex.\textsuperscript{19,20} These genes affect the enzyme glyoxalase-1 and A-chain of \(\alpha\)-crystallin, respectively. The given haplotypes of RT1 complex are associated with susceptibility or resistance to certain diseases (eg, insulin-dependent diabetes mellitus).\textsuperscript{21}

It has been demonstrated that the immune system of spontaneously hypertensive rats (SHR) is depressed and the chronic phase of hypertension is thymus-dependent.\textsuperscript{22} Not only were fewer T lymphocytes reported in adult SHR rats but there was even a significant decline of this cell population during aging in contrast to Wistar-Kyoto rats in which the number of T lymphocytes increased with age. It was also noted that the functional capability of T lymphocytes in SHR was depressed. Moreover, manipulation of the immune system with immunosuppressive treatment attenuated hypertension development in SHR.\textsuperscript{23} Recently, immune system abnormalities were disclosed even in 2-week-old SHR rats, favoring the hypothesis that the immunologic disturbances are of a primary nature.\textsuperscript{24}

The immune system is also implicated in the cause of hypertension in other animal models of spontaneous hypertension. Neonatal removal of thymus attenuated hypertension development in Lyon hypertensive rats.\textsuperscript{25} New Zealand Black (NZB) mice developed spontaneous hypertension,\textsuperscript{26} but their substrain, which is genetically athymic (nude NZB), remained normotensive. Immunosuppressive therapy with cyclophosphamide normalized blood pressure in NZB mice. Moreover, grafting of thymus tissue from NZB mice into nude NZB ones induced hypertension.\textsuperscript{26}

It was demonstrated by use of recombinant inbred (RI) strains that genes within the RT1 complex or closely linked to it are associated with blood pressure.\textsuperscript{27} In addition, we have found that the polymorphism of the \textit{hsp70} gene between normotensive and hypertensive rats was associated with a blood pressure difference of 15 mm Hg in RI strains.\textsuperscript{28} This was not revealed by Lodwick et al.,\textsuperscript{29} who found no evidence of linkage between blood pressure and the \textit{hsp70} gene locus or other genes located within the RT1 complex. The reasons for the discrepant findings are unclear, but they could reflect the use of different control strains or different techniques of blood pressure monitoring.

The aim of this study was to evaluate further the involvement of the genes of the rat's major histocompatibility complex (RT1 complex) in relation to blood pressure levels and blood pressure response to stress. The combination of inbred strain with its congenic partners and with its recombinant lines is an ideal tool for studying the influence of one particular chromosomal segment on the same genetic background.

**METHODS**

We used adult (6-month-old) male Lewis normotensive rats (LEW, I haplotype of RT1 complex) as well as inbred congenic strains LEW.1A (RT1 \(^a\)) and LEW.1W (RT1 \(^{\text{w}}\)) in which the RT1 complex of AVN (a haplotype) and Wistar Prague (u haplotype) was transferred to Lewis genetic background by repeated backcrossing.\textsuperscript{30} In addition, recombinant lines LEW.1AR1 (RT1\textit{arl}, r2), LEW.1AR2 (RT1\textit{ar2}, r3), LEW.1WR1 (RT1\textit{wr1}, r4), and LEW.1WR2 (RT1\textit{wr2}, r6)\textsuperscript{31-33} were used in this study. The recombinant haplotypes of the RT1 complex were originally detected during combined serologic and histogenetic screening program of segregating hybrids derived from RT1 congenic parental strains LEW.1A and LEW.1W.\textsuperscript{31-33} All six inbred strains were bred by brother–sister mating in Prague and were routinely checked for isohistogenility by intrainfamilial skin graft exchange. They represent the full haplotypes RT1\(^a\) and RT1\(^{w}\) and recombinant haplotypes between them enabling us to distinguish particular regions of the RT1 complex (Table 1) (for review see ref. 34). A molecular genetic approach made possible a more precise description of regions in the rat RT1 complex. The RT1.A region codes the classic class I antigens, the RT1.B/D region determines the class II antigens, and the RT1.C region encodes the class I-like antigens.\textsuperscript{35}

All animals were maintained in air-conditioned facilities, at constant temperature (23°C), with a 12-h light-dark cycle, on a normal rat chow and with free access to tap water.

Blood pressure of conscious animals was measured by tail-cuff plethysmography immediately after placing the animals into a holder without previous training. The mean of five readings was taken as final blood pressure value, which corresponds to moderate restraint stress. The following day, systolic, mean arterial, and diastolic blood pressures were measured under light ether anesthesia using P23 Db Statham transducer (Valley View, OH) and Hewlett-Packard recorder (Andover, MA). Animals were killed and the weights of the hearts and kidneys were determined.

All data were expressed as means ± SEM. Statistical analysis was done using Student's \(t\) test or one-way analysis of variance followed by the calculation of
least significant differences. Values of $P < .05$ were considered as significant.

RESULTS

Table 2 summarizes the body weight, relative organ weights, as well as systolic blood pressure during moderate restraint stress in all strains studied. Body weight was comparable in all strains except the congenic strain LEW.1W and the recombinant line LEW.1WR1. During restraint, stress systolic blood pressure of the congenic strain LEW.1W was significantly lower in comparison with the Lewis strain. Systolic blood pressure of all recombinant lines was elevated compared to the respective congenic strains, three of them having even significantly higher blood pressure than Lewis rats (Table 2). Both relative heart and kidney weights were significantly lower in almost all strains when compared to Lewis animals. The only exception was recombinant line LEW.1AR2, which did not differ from Lewis rats.

Systolic blood pressure measured under light ether anesthesia was similar in Lewis and both congenic strains (Figure 1). The only blood pressure elevation was observed in recombinant line LEW.1WR2 in comparison with either Lewis, LEW.1W, or LEW.1AR2 rats (Figure 1). The same was true for diastolic blood pressure.

DISCUSSION

Results of this study confirmed our previous suggestion that the genes of MHC of the rat (RT1 complex) might be involved in blood pressure regulation. Moreover, the involvement of the genes within the RT1 complex may be relevant for the environmental response implicated in the development of hypertension. As discussed in the introduction, genes predominantly in the class III region could be involved in this process, but there is still high probability that the genes of class I or II are also involved. However, because of the linkage disequilibrium displayed by alleles within the MHC, it is not clear in many cases whether the increased susceptibility is due to the products of particular class I, II, or III loci per se, due to a combination of alleles at various loci, or due to the products of genes not yet identified.

Recently we have shown the association between RT1 complex and relative heart weight. Our present study demonstrates the different impact of individual RT1 haplotypes on both relative heart and kidney weight. Because of the same genetic background it

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**TABLE 1. RT1 COMPLEX AND RECOMBINANT SITES OF VARIOUS RT1 HAPLOTYPES**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Haplotype</th>
<th>Class I RT1.A</th>
<th>Class I RT1.B/D</th>
<th>Class III C4</th>
<th>Class III Bf</th>
<th>Class III Hsp</th>
<th>Class I-like RT1.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEW</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LEW.1A</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>LEW.1W</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>LEW.1AR1</td>
<td>r2</td>
<td>a</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>LEW.1AR2</td>
<td>r3</td>
<td>a</td>
<td>a</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>LEW.1WR1</td>
<td>r4</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>u</td>
</tr>
<tr>
<td>LEW.1WR2</td>
<td>r6</td>
<td>u</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

Data are derived from refs. 12, 17, and 35. The vertical bars indicate the recombinant sites.

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**TABLE 2. BODY WEIGHT (BW), RELATIVE HEART (HW/BW), AND RELATIVE KIDNEY (KW/BW) WEIGHTS AS WELL AS INDIRECT SYSTOLIC BLOOD PRESSURE (SBP) MEASURED DURING MODERATE RESTRAINT STRESS IN ALL STRAINS STUDIED**

<table>
<thead>
<tr>
<th>Strain (n)</th>
<th>BW (g)</th>
<th>HW/BW (mg/100 mg BW)</th>
<th>KW/BW (g)</th>
<th>SBP$_{in}$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis (n = 12)</td>
<td>330 ± 7</td>
<td>242 ± 2</td>
<td>650 ± 1</td>
<td>112 ± 3</td>
</tr>
<tr>
<td>LEW.1A (n = 9)</td>
<td>349 ± 9</td>
<td>218 ± 4*</td>
<td>579 ± 8*</td>
<td>110 ± 2</td>
</tr>
<tr>
<td>LEW.1AR1 (n = 11)</td>
<td>322 ± 9</td>
<td>217 ± 5*</td>
<td>575 ± 12*</td>
<td>124 ± 1*</td>
</tr>
<tr>
<td>LEW.1AR2 (n = 11)</td>
<td>340 ± 5</td>
<td>233 ± 5‡</td>
<td>637 ± 13‡</td>
<td>137 ± 1*</td>
</tr>
<tr>
<td>LEW.1W (n = 10)</td>
<td>360 ± 4*</td>
<td>218 ± 2*</td>
<td>624 ± 5†</td>
<td>92 ± 1*</td>
</tr>
<tr>
<td>LEW.1WR1 (n = 11)</td>
<td>372 ± 8*</td>
<td>212 ± 2*</td>
<td>610 ± 7†</td>
<td>111 ± 1†</td>
</tr>
<tr>
<td>LEW.1WR2 (n = 11)</td>
<td>340 ± 5</td>
<td>228 ± 3‡</td>
<td>608 ± 14*</td>
<td>131 ± 1*†</td>
</tr>
</tbody>
</table>

*P < .01 significantly different from Lewis rats; †P < .05 significantly different from the respective congenic strain. (LEW.1A or LEW.1W).
could be speculated that the genes within \( a \) and \( u \) haplotypes can decrease relative heart and kidney weight in comparison with the \( l \) haplotype. The use of recombinant lines further revealed that the gene-to-gene interaction inside the RT1 complex might be very important because the second recombination increased relative heart weight in both recombinant lines (LEW.1AR2 and LEW.1WR2), whereas relative kidney weight was increased only in LEW.1AR2. The candidate genes involved in heart and kidney weight determination might be searched for predominantly within the growth and reproduction complex that is located in the RT1 complex.\textsuperscript{39}

There are several known genes in the RT1 complex with potential cardiovascular implications (including those for tumor necrosis factor-\( \alpha \), 21-hydroxylase, and \( hsp70 \)), but there could be many as yet not identified genes. The impact of the genes of the RT1 complex on blood pressure was recognized in the set of recombinant inbred (RI) strains.\textsuperscript{27} In the same set of RI strains we have found that the \( hsp70 \) restriction fragment length polymorphism was associated with a blood pressure difference of 15 mm Hg.\textsuperscript{28} This was not revealed in the study by Lodwick et al.\textsuperscript{29} in SHR \( \times \) Wistar-Kyoto crosses who found no linkage between the \( hsp70 \) gene locus or other genes located within the RT1 complex and blood pressure. These researchers discussed several explanations for these discrepant results. One of them was that the genetic background provided by Lodwick's cross may have prevented the expression of the effect of the SHR-specific locus (in this case \( hsp70 \) locus) on blood pressure. The results of our study support this explanation. The use of congenic strains with their recombinant lines suggested that the gene-to-gene interaction can play a significant role in the expression of genes. It was evident that even on "normotensive" genetic background the interaction of certain gene regions within the RT1 complex (recombinant LEW.1WR2 line) led to blood pressure increase not only during moderate restraint stress but even under light ether anesthesia. Recently we have found that genes within or close to the RT1 complex are partially responsible for the salt sensitivity of the rat.\textsuperscript{40} The more detailed genetic analysis of the RT1 complex including its interaction with other genes throughout the genome would be necessary to clarify its role in the control of blood pressure level.

In conclusion, our results confirmed our previous observations about the significant role of the RT1 complex in blood pressure regulation. Moreover, it is evident that at least in some genetic "combinations" the genes of the MHC might be implicated in hypertension development per se or in higher susceptibility to environmental stimuli. The further analysis of these mechanisms might be rather difficult due to the genetic complexity that includes multiple alleles, differences in genetic background, linkage relationships, and genotype-environment interaction.\textsuperscript{41} The net effect of these factors makes many results strain- and cross-specific. Therefore, it would be naive to expect comparable blood pressure effects of particular loci under various experimental conditions.

REFERENCES


