Vol. 188, No. 1, 1992
October 15, 1992
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 64-69

FURTHER EVIDENCE OF THE SA GENE AS A CANDIDATE GENE CONTRIBUTING TO THE HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RAT

Naoharu Iwai*, Theodore W. Kurtz**, and Tadashi Inagami

Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232

Received August 31, 1992

Summary: We have recently reported that the allele of the SA gene of the Spontaneously hypertensive rat (SHR) has a capacity to influence blood pressure in a F2 rat population prepared from SHR and Wistar-Kyoto rat. In the present study, we have undertaken a similar genetic co-segregation analysis of the F2 rat population prepared from SHR and Lewis rat. The result indicated that, although overall effects of the SA gene genotypes on blood pressure were not significant, a correlation of the genotypes of the SA gene with blood pressure was significantly observed in the female rats of this population. The present results further strengthen our hypothesis that the SA gene, or a gene closely linked to this gene, has a capacity to influence blood pressure.

Kidney plays a central role in the pathogenesis of hypertension in Spontaneously Hypertensive rat (SHR)(1,2,3). Based on the assumption that some of the genes contributing to the hypertension in SHR might be differentially expressed between the kidneys of SHR and its normotensive control Wistar-Kyoto rat (WKY), three genes have been isolated as candidate genes for SHR hypertension (4). One of them, designated as SA, is most interesting because the expression level of this gene in the SHR kidney is more than ten times higher than that in the WKY kidney from the 4th week of age (4). However, this differential expression might be a result of hypertension or just a strain difference nothing to do with the hypertension. To clarify this issue, we have performed a genetic co-segregation analysis in a F2 rat population prepared from SHR and WKY, and a co-segregation of the SHR allele of the SA gene with higher blood pressure was observed in that population (5).

* Present Address: The 1st Department of Internal Medicine, Shiga University of Medical Science, Seta, Ohtsu 520-21, Shiga-ken, Japan.

**Department of Laboratory Medicine, UCSF Medical Center, San Francisco, CA.

0006-291X/92 $4.00

Multiple genes seem to be involved in the pathogenesis of hypertension in SHR(6). This means that a genetic background is important for a gene to express its effects on blood pressure (6). In this communication, we have analysed a correlation of the genotypes of the SA gene with blood pressure in a F2 rat population prepared from SHR and Lewis rat which has a different genetic background from the one analysed previously. Moreover, differential expression of the SA gene in the brain between SHR and other normotensive strains of rat was analysed.

Materials and Method

Animals

Male spontaneously hypertensive rat (SHR), Wistar-Kyoto rat (WKY), and Lewis rat were purchased from Charles River Laboratories, Inc. (Wilmington, MA, USA). The F2 rats analysed in the present study comprised of 85 male and 84 female rats prepared from SHR and Lewis rat as previously described (7). At 22-26th week of age, mean arterial pressures were directly measured in unanesthetised and unrestrained state as previously described (7).

Assessment of the expression levels of the SA gene in various tissues

A competitive polymerase chain reaction method(8,9) was emoployed to reassess the expression levels of the SA gene in other tissues than kidneys. The Xba I (97)-Msc I (961) fragment of the SA cDNA (4) was subcloned into pBluescript II KS(+) (Stratagene, La Jolla, CA, USA), designated as pSA. The pSA was digested by the restriction enzyme Nsi I and Nco I, was blunt ended by the Klenow treatment, and was self ligated. The resultant plasmid contained a cDNA fragment which lacked the region between the Nsi I (177) and Nco I (287) sites. This plasmid was linealized with Xho I and the deletion mutated cRNA was synthesized by T7 RNA polymerase. Ten micrograms of total RNAs from various tissues and the deletion mutated cRNA of known amounts were combined, and were reverse-transcribed, as previously described (9). The resultant cDNA mixtures were amplified by the polymerase chain reaction method using the following oligonucleotides as primers:

5'-GAAGAGCTCAAGATCACTGACTTGTGAGC-3' (101-130)
5'-CAGGGAACAGGCTTCTGTGAGTATGTTGGC-3' (621-592).

The amplification profile included 30cycles of denaturation at 95°C for 30sec., annealing at 57°C for 60 sec., and polymerase reaction at 74°C for 120 sec. The amplification of the SA mRNA and the deletion mutated cRNA by these primers should give 521bp and 411bp fragment, respectively. Total RNA was isolated as previously described (4).

Southern blot analysis.

Southern blot analysis was performed as previously described (4).

Statistical analysis.

One way and two way ANOVA analyses were performed using a software "StatView II " (Abacus Concepts, Inc., Berkley, CA, USA).

Results

Expression of the SA gene in the Central nervous system

The expression level of the SA gene in the brains of WKY was found to be more than ten times higher than those of SHR from the 4th week of age. Fig. 1 shows an analysis of 8 week old SHR and WKY.

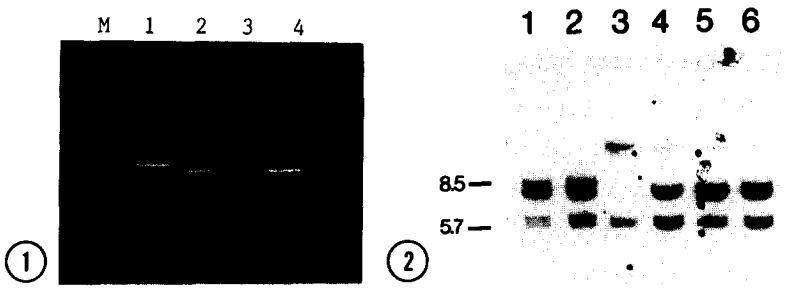

Figure 1. Expression of the SA gene in the brain
Ten micrograms of the brain total RNA from the 8 week old SHR was combined with 20fg (lane 1) and 200fg (lane 2) of the deletion mutated RNA, while ten micrograms of the brain total RNA from the age-matched WKY was combined with 2 pg (lane 3), and 20pg (lane 4) of the deletion mutated RNA. Those RNA mixtures were reverse transcribed, and the resultant cDNA mixtures were amplified by PCR. The M lane indicates a size marker of Phi-X DNA digested with Hae III.

Figure 2. Genetic polymorphisms of the SA gene
Ten micrograms of the high molecular weight DNA isolated from various strains of rat was digested with a restriction enzyme Stu I and was resolved on 0.7% agarose gel. Lane 1. Brown-Norway rat, Lane 2. Fisher rat, Lane 3. SHR, Lane 4. F1 rat from SHR and WKY, Lane 5. WKY, Lane 6. Lewis rat.

Co-segregation analysis of the genotype with blood pressure

The expression level of the SA gene in the kidney of Lewis rat is almost comparable to that of WKY, and that in the brain of Lewis rat is also comparable to that of WKY (data not shown). Moreover, the Southern blot pattern of the Lewis rat obtained by the restriction enzyme Stu I is indistinguishable from that of WKY (Fig. 2). The genotype of each F2 rat was determined by using this Stu I restriction fragment polymorphism.

Table 1 and Fig. 3 show a summary obtained from the F2 rat population employed in the present study. Although the overall effects of the SA genotype on blood pressure were not significant ($p=0.169$), a significant effect ($p=0.021$) was obtained for sex(A) X genotype(B) interaction. On subgroup analysis, the effects of the SA genotype on blood pressure were observed only in the female rats of this F2 rat population ($p=0.011$ by one way ANOVA and $p=0.007$ by Kruskal-Wallis test). Subgroup analysis of the female F2 rats showed that the blood pressure values of the rats inheriting two SHR alleles of the SA gene were significantly higher ($p<0.015$ by Scheffe F-teat) than those of the rats inheriting two Lewis alleles of the SA gene.

Discussion

The SA gene is differentially expressed between SHR and normotensive strains of rat, namely WKY, and Lewis rat. In the present study, we have found that the expression levels of the SA gene in the brains of the normotensive strains of rat were

Table 1. Summary of the genotype-phenotype analysis

	STATISTICS BY GENOTYPE(mmHg)		
	SS	SL	LL
MALE	120.8±2.1 n=24	122.2±2.1 n=42	122.8±2.2 n=19
FEMALE	133.5±2.1 n=23	127.6±1.8 n=45	122.6±2.1 n=15

LL, SL, and SS indicate genotypes homozygous for Lewis alleles , heterozygous, and homozygous for SHR alleles of the SA gene, respectively. Statistical details are described in the text.

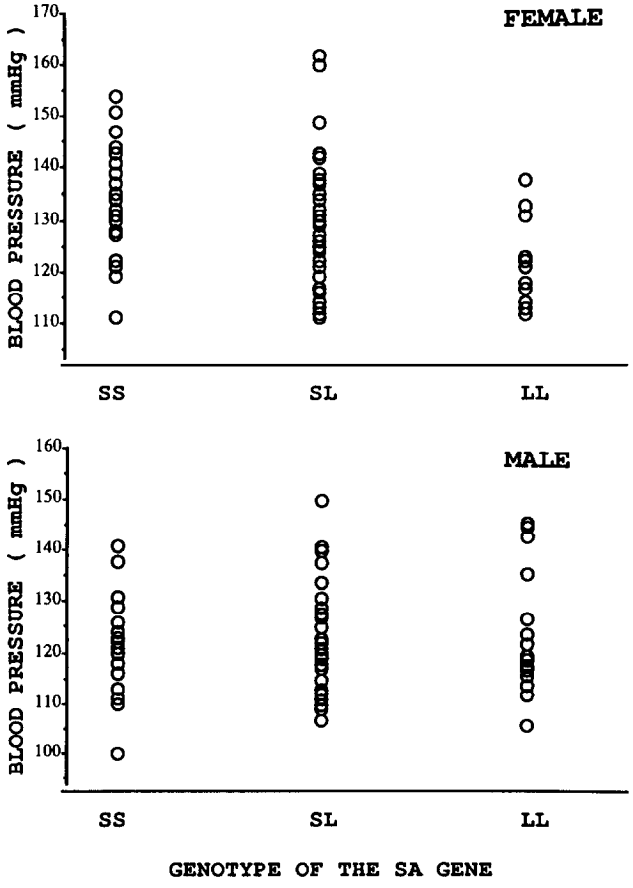

Figure 3. Blood pressure values of the F2 rats
Blood pressure values of the F2 rats are shown according to their genotype of the SA gene. Phenotypic data are summarized in Table 1.

higher than that in the brains of SHR from the 4th week of age. Although differential expressions are intriguing, these findings are not enough to conclude that the SA gene is a gene contributing to the hypertension in SHR. To clarify the issue whether the SA gene is really a gene contributing to the hypertension in SHR, we have analysed whether the SHR allele of the SA gene co-segregates with higher blood pressure in a F2 rat population prepared from SHR and WKY (4). The result indicated that the blood pressure values of the rats inheriting two SHR alles of the SA gene had higher blood pressure values than those of the rats inheriting two WKY alleles of the SA gene ($p < 0.005$ Scheffe-F test) (4).

Multiple genes seem to be involved in the pathogenesis of SHR hypertnsion (6), which means that genetic background is important for a gene to express its effects on blood pressure. In this sense, it would be interesting to see the effects of the genotype of the SA gene on blood pressure in F2 rat populations of different genetic backgrounds. The F2 rat population employed in the present study was originated from SHR and Lewis rat, which should have a different genetic background from the one analysed previously. For instance, the blood pressure values of the male rats of this F2 population were lower than those of the female rats, which was not observed in F2 rat populations prepared from SHR and WKY (10).

Although overall effects of the genotype of the SA gene on blood pressure were not observed in this population, in the female F2 rat population, a correlation of the genotypes of the SA gene with blood pressure was significantly observed . From these results, we can conclude that the SA gene or a gene closely linked to this gene has a capacity to influence blood pressure and the effects of the SHR allele of the SA gene on blood pressure depend on a genetic background of an analysed population.

The fuctions of the SA gene product are unknown. Our recent analysis revealed that the expression levels of the SA gene in the brain, but not in the kidney, correlated well with the genotypes of the SA gene (11). This might indicate functions of the SA gene product in the brain, but not in the kidney is important for this gene to influence blood pressure. Further study to identify functions of this gene product and an examination of a possible involvement of this gene in human primary hypertension are the nex problems.

References

1. Kawabe K, Watanabe T, Shiono K, and Sokabe H. (1978) Jpn.Heart J. 19, 886-894
2. Rettig R, Strauss H, Folberth C, Ganten D, Waldherr R, and Unger T. (1989) Am.J.Phisiol. 267, F197-F203
3. Guyton AC. (1987) Hypertension 10, 1-6

4. Iwai N, and Inagami T. (1991) Hypertension 17, 161-169
5. Iwai N, and Inagami T. J.Hypertens. (in press).
6. Rapp JP (1991) Hypertension 18, I-18 - I-28
7. Kurtz TW, Simonet L, Kabra PM, Wolfe S, Chan L, and Hjelle BL. (1990) J.Clin.Invet. 85, 1328-1332
8. Wang AM, Doyle MV, and Mark DF. (1989) Proc.Natl.Acad.Sci.USA 85, 9717-9721
9. Iwai N, and Inagami T. (1992) Biochem.Biophys.Res.Commun.. 182, 1094-1099
10. Tanase H. Exp.Anim. (1979) 28, 519-530
11. Iwai N, Chaki S, and Inagami T. (1992) in ' Genetic Hypertension' edited by J. Sassard. Colloque INSERM/John Libbey Eurotext Ltd. Vol. 218, pp 301-303