Species-Specific Kinetics of Mouse Renin Contribute to Maintenance of Normal Blood Pressure in Transgenic Mice with Overexpressed Human Angiotensinogen

Shigeru TAKAHASHI, Akiyoshi FUKAMIZU*, Toshihisa HATAE, Yuji YAMADA, Fumihiro SUGIYAMA¹⁾, Noriko KAJIWARA¹⁾, Ken-ichi YAGAMI¹⁾, and Kazuo MURAKAMI

Institute of Applied Biochemistry and ¹⁾Laboratory Animal Research Center, University of Tsukuba, Tsukuba, Ibaraki 305, Japan
(Received 19 February 1992/Accepted 11 July 1992)

ABSTRACT. Angiotensinogen, the precursor of a vasoactive octapeptide angiotensin II, is the only known natural substrate of renin, and its reaction exhibits strict species specificity and is the rate-limiting step in the renin-angiotensin system that controls the blood pressure. We measured blood pressure and heart rate of the transgenic mice with overproduced human angiotensinogen, and showed no significant difference in these parameters between transgenic and nontransgenic mice. We also provided evidence that mouse renin could not cleave human angiotensinogen, indicating a lack of angiotensin production from the human substrate. These results suggested that the blood pressure of transgenic mice is normally maintained, probably due to the inability of mouse renin to release angiotensin from the transgene products.—**KEY WORDS:** blood pressure, renin-angiotensin system, transgenic mouse.

J. Vet. Med. Sci. 54(6): 1191–1193, 1992

Angiotensinogen is a moderately abundant 55–60 kDa serum glycoprotein that is the precursor of the angiotensin peptides and is the only known naturally occurring substrate of renin, an aspartyl proteinase [20]. The substrate is synthesized by a variety of cells, most predominantly by the liver, and it is constitutively secreted into the circulation as the protein is translated from its mRNA [15]. Renin is produced by the kidney and secreted into the blood flow, where it cleaves angiotensinogen to yield a decapeptide angiotensin I. Subsequently, by angiotensin-converting enzyme, this peptide is processed to an octapeptide angiotensin II, which causes anterior vasoconstriction and stimulates aldosterone release from the adrenal gland. Through the synthesis of angiotensin peptide, the mammalian renin-angiotensin system has long been believed to play an important role in the control of blood pressure and the regulation of electrolyte balance, and possibly in the pathogenesis of hypertension [20].

For the study of high blood pressure, hypertensive rats [16] and mice [21] have been genetically selected and widely used as excellent model animals. On the other hand, a number of transgenic animals carrying genes for the components of the renin-angiotensin system have been generated [2, 4–6, 13–15, 25], since the development of techniques to introduce eukaryotic gene sequences into the genome of animal fertilized eggs has opened a field of research requiring the collaboration of molecular biology and animal physiology [3, 18]. By using this new technique, we have previously generated the transgenic mice with overexpressed human angiotensinogen, demonstrated the tissue-specific expression of the introduced gene in the transgenic mice [23], and examined the effect of inflammation on expression of the endogenous and exogenous angiotensinogen genes of these mice [24]. However, we have not investigated the blood pressure level of these transgenic mice. We have not also examined the substrate specificity of mouse renin, although the

reaction of renin and angiotensinogen is believed to be the rate-limiting step in the renin-angiotensin system and to exhibit strict species specificity. In the present report, we measured the blood pressure of transgenic mice carrying the human angiotensinogen gene and characterized the biochemical property of mouse renin to the human substrate by an *in vitro* assay system.

In order to assess the physiological consequence of the overexpression of human angiotensinogen in the transgenic mice, we have examined systolic blood pressure level of six transgenic male mice and six normal male siblings in the F₂ generation. The blood pressure and heart rate were measured with a programmable sphygmomanometer (PS-200; Riken Kaihatsu, Machida, Japan) using tail cuff methods, connected to a pulse amplifier. The unanesthetized mice were placed in metal holder and warmed at 32°C before measurement. Five readings were taken for each measurement and the data were expressed as the mean $\pm$ standard deviation. As summarized in Table 1, there was no definite difference in the systolic blood pressure between transgenic and nontransgenic littermates. Heart rate of the transgenic mice was also indistinguishable from that of nontransgenic ones. These results showed that the blood pressure is maintained at physiologically normal states in the transgenic mice despite the overexpression of human angiotensinogen.

Although several reasons can be considered why the blood pressure is not elevated in such a transgenic mouse

Table 1. Blood pressure and heart rate of siblings obtained by mating between the normal and transgenic mice

Mouse	Blood pressure (mmHg)	Heart rate (beats/min)
NM ^{a)}	98.1 $\pm$ 5.6	615.3 $\pm$ 55.1
TgM ^{b)}	97.0 $\pm$ 7.8	643.6 $\pm$ 69.2
p	NS ^{c)}	NS

Values of blood pressures are expressed as the mean $\pm$ SD. Six male mice from each group were successfully measured for mean systolic blood pressure and heart rate. Statistical significance between the two groups was determined by Student's *t* test. a) NM, normal mice. b) TgM, transgenic mice c) NS, not significant.

* CORRESPONDENCE TO: FUKAMIZU, A., Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

Fig. 1. Substrate specificity of mouse renin. Mouse renin purified from submaxillary gland (mSMGRN) or recombinant human renin (rhRN) (1 to 4 ng) were incubated with either porcine angiotensinogen (pANG) (100 nM) or recombinant human angiotensinogen (rhANG) (30 nM) at 37°C for 20 min, pH 7.4 for rhANG or pH6.5 for pANG, in the presence of proteinase inhibitors, 5 mM phenylmethanesulphonyl fluoride and 5 mM EDTA. Renin activity was measured as the rate of angiotensin I formation and the formed angiotensin I was quantified by radioimmunoassay [7].

with the overexpressed angiotensinogen, it is possible that mouse renin could not react with the introduced human substrate. To test this possibility, we examined whether mouse renin has an ability to generate angiotensin I from human substrate *in vitro* by radioimmunoassay [7]. We used mouse renin purified from submaxillary gland (SMG) [22] for the study of this substrate specificity, because SMG renin was immunologically and physicochemically similar to renal renin [12]. As a reaction control, we used recombinant human renin purified from a Chinese hamster ovary cell line, rh-renin [19].

For the *in vitro* assay, porcine angiotensinogen (pANG) was partially purified from plasma, since it was easy to prepare and widely used as a substrate to the measurement of mammalian renin activity, including mouse [22], rat [11], and human [26]. A conditioned medium was prepared by culturing a clonal Chinese hamster ovary cell line expressing human angiotensinogen, which is referred to as recombinant human angiotensinogen (rhANG) [9]. As demonstrated in Fig. 1, the SMG renin and rh-renin showed angiotensin I-generating activities, when used pANG as a substrate. On the other hand, mouse renin was not capable of cleaving rhANG, although rh-renin produced angiotensin I from the homologous substrate efficiently. These findings suggested that mouse renin is very inefficient in catalyzing hydrolysis of the overexpressed human substrate in the transgenic mice.

The physiological phenotype of the transgenic mice was unaltered despite of markedly expressed human angiotensinogen; there was no difference in the blood pressure and heart rate between transgenic and nontransgenic groups. Angiotensinogen is thought to be constitutively secreted into the circulation soon after synthesis [15]. It is therefore conceivable that the transgene products synthesized by the

liver are circulating in the plasma, although we have not tested the plasma level of angiotensinogen in the transgenic mice. Considering that cleavage of angiotensinogen by renin is the rate-limiting step in the renin-angiotensin system, the formation of angiotensin I by this reaction seems to be an important process for elevation of blood pressure. Thus, if mouse renin could not cleave human angiotensinogen, it is reasonable that blood pressure is maintained at the normal baseline in the transgenic mice.

It has been shown that the action of renin on its substrate is dependent on the species from which these substances are derived [1, 17]. For example, mouse renin is capable of cleaving angiotensinogen derived from mouse, rat, rabbit, porcine, canine, bovine, and goat, whereas renin derived from human, rat, hamster, rabbit, and porcine does not have an ability to cleave mouse angiotensinogen. However, there has been no report with respect to the reaction of mouse renin and human angiotensinogen. Our present study is the first demonstration that mouse renin is very inefficient in catalyzing hydrolysis of human angiotensinogen. Thus, one plausible explanation for our transgenic mice is that the maintenance of normal blood pressure in mice with overexpressed human angiotensinogen is due to the inability of mouse renin to cleave the human substrate.

A less likely alternative is that a desensitization of angiotensin receptors or feedback regulation by some hypotensive systems, such as atrial natriuretic peptide and the kinin-kallikrein system, may occur owing to the overproduction of angiotensinogen. Such circumstances of metabolic compensation in response to excessive circulating levels of potent neuroendocrine peptides were found in transgenic mice expressing vasopressin [8] or somatostatin [10] genes. In spite of blood levels of somatostatin and vasopressin that were 100–1000 times and 25–500 times elevated above normal levels, respectively, these mice were similarly free of any grossly detectable physiologic abnormalities.

Our present study on physiological state in transgenic mice with overexpressed human angiotensinogen and on the biochemical property of renin-angiotensinogen reaction should provide basic information for further characterization of species-specific kinetics of renin and its substrate. In addition, the transgenic mice carrying the gene for human angiotensinogen with species-specific reactivity to renin may take advantage of producing an animal model of human-type hypertension by mating with the transgenic mice harboring the human renin gene, whose expression is regulated in a cell-specific manner [4–6].

ACKNOWLEDGEMENTS. We wish to thank Professor Motoya Katsuki (Department of Molecular and Cellular Biology, Institute of Bioregulation, Kyushu University) and his research group (Central Institute for Experimental Animals) for their helpful instruction for the generation of the transgenic mice. This work was supported by grants from the Ministry of Education, Science, and Culture of Japan, Chichibu Cement Co., Ltd., and Special

Research Project on Circulation Biosystems in University of Tsukuba.

REFERENCES

1. Burton, J. and Quinn, T. 1988. *Biochim. Biophys. Acta* 952: 8–12.
2. Clouston, W. M., Lyons, I. G., and Richards, R. I. 1989. *EMBO J.* 8: 3337–3343.
3. Connelly, C. S., Fahl, W. E., and Iannaccone, P. M. 1989. *Exp. Cell Res.* 183: 257–276.
4. Fukamizu, A., Seo, M. S., Hatae, T., Yokoyama, M., Nomura, T., Katsuki, M., and Murakami, K. 1989. *Biochem. Biophys. Res. Commun.* 165: 826–832.
5. Fukamizu, A., Hatae, T., Kon, Y., Sugimura, M., Hasegawa, T., Yokoyama, M., Nomura, T., Katsuki, M., and Murakami, K. 1991. *Biochem. J.* 278: 601–603.
6. Fukamizu, A., Uehara, S., Sugimura, K., Kon, Y., Sugimura, M., Hasegawa, T., Yokoyama, M., Nomura, T., Katsuki, M., and Murakami, K. 1991. *J. Biol. Regul. Homeost. Agents* 5: 112–116.
7. Haber, E., Koerner, T., Page, L. B., Kilman, B., and Purnode, A. 1969. *J. Clin. Endocrinol.* 29: 1349–1355.
8. Habener, J. F., Cwikel, B. J., Hermann, H., Hammer, R. E., Palmiter, R. D., and Brinster, R. L. 1989. *J. Biol. Chem.* 264: 18844–18852.
9. Hatae, T., Takimoto, E., Fukamizu, A., Hori, K., Kimoto, K., and Murakami, K. 1992. *Biochim. Biophys. Acta* 1121: 335–338.
10. Low, M. J., Hammer, R. E., Goodman, R. H., Habener, J. F., Palmiter, R. D., and Brinster, R. L. 1985. *Cell* 41: 211–219.
11. Matoba, T., Murakami, K., and Inagami, T. 1978. *Biochim. Biophys. Acta* 526: 560–571.
12. Michelakis, A. M., Yoshida, H., Menzie, J., Murakami, K., and Inagami, T. 1974. *Endocrinology* 94: 1101–1105.
13. Mullins, J. J., Sigmund, C. D., Kane-Haas, C., and Gross, K. W. 1989. *EMBO J.* 8: 4065–4072.
14. Mullins, J. J., Peters, J., and Ganten, D. 1990. *Nature (Lond.)* 344: 541–544.
15. Ohkubo, H., Kawakami, H., Kakehi, Y., Takumi, T., Arai, H., Yokota, Y., Iwai, M., Tanabe, Y., Masu, M., Hata, J., Iwao, H., Okamoto, H., Yokoyama, M., Nomura, T., Katsuki, M., and Nakanishi, S. 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 5153–5157.
16. Okamoto, K. and Aoki, K. 1963. *Jpn. Circul. J.* 27: 282–293.
17. Oliver, W. J. and Gross, F. *Proc. Soc. Exp. Biol.* 122: 923–926.
18. Palmiter, R. D. and Brinster, R. F. *Annu. Rev. Genet.* 20: 465–499.
19. Poorman, R. A., Palermo, D. P., Post, L. E., Murakami, K., Kinner, J. H., Smith C. W., Reardon, I., and Henrikson, R. L. 1986. *Proteins: Structure Function Genetics* 1: 139–145.
20. Reid, I. A., Morris, B. J., and Ganong, W. F. 1978. *Annu. Rev. Physiol.* 40: 377–410.
21. Schlager, G. 1976. *Genetics* 76: 537–549.
22. Suzuki, F., Nakamura, Y., Nagata, Y., Ohsawa, T., and Murakami, K. 1982. *J. Biochem. (Tokyo)* 89: 1107–1112.
23. Takahashi, S., Fukamizu, A., Hasegawa, T., Yokoyama, M., Nomura, T., Katsuki, M., and Murakami, K. 1991. *Biochem. Biophys. Res. Commun.* 180: 1103–1109.
24. Takahashi, S., Fukamizu, A., Sugiyama, F., Kajiwar, N., Yagami, K., and Murakami, K. 1992. *J. Vet. Med. Sci.* 54: 367–369.
25. Tronik, D., Dreyfus, M., Babinet, C., and Rougeon, F. 1987. *EMBO J.* 6: 983–987.
26. Yokosawa, H., Holladay, L. A., Inagami, T., Haas, E., and Murakami, K. 1980. *J. Biol. Chem.* 255: 3498–3502.