BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **242,** 238–243 (1998)
ARTICLE NO. RC977800

# A Novel Vascular Modulator, Hepatocyte Growth Factor (HGF), as a Potential Index of the Severity of Hypertension

Shigefumi Nakamura, Atsushi Moriguchi, Ryuichi Morishita, Motokuni Aoki, Yoshikage Yo, Shin-ichiro Hayashi, Nobuaki Nakano, Tomohiro Katsuya, Sachiko Nakata, Seiju Takami, Kunio Matsumoto,* Toshikazu Nakamura,* Jitsuo Higaki, and Toshio Ogihara

*Department of Geriatric Medicine, Osaka University Medical School, Suita 565, Japan; and *Division of Biochemistry, Department of Oncology, Biomedical Research Center, Osaka University Medical School, Suita 565, Japan*

Received November 10, 1997

**HGF (hepatocyte growth factor), a member of endothelium-specific growth factors, might contribute to protection and/or repair of vascular endothelial cells injured by high blood pressure (BP). If so, serum HGF level might be elevated in response to endothelial cell damage. To test this hypothesis, we measured serum levels of HGF in hypertensive and normotensive patients. Serum HGF concentration in hypertensive patients without any complication was significantly higher than normal subjects ($p<0.001$). Serum HGF concentration showed a significant positive correlation with BP ($p<0.01$). Interestingly, serum HGF concentration in hypertensive patients with complications was significantly higher than that in hypertensive patients without complication and normotensive subjects ($p<0.01$). Of importance, hypertensive patients treated with antihypertensive drugs showed the same level of serum HGF concentration as normotensive subjects ($p<0.001$). The present study demonstrated that serum concentration of HGF is significantly elevated dependent on the severity of hypertension, suggesting that HGF may be a new index of the severity of hypertension.** 

***Key Words:*** **endothelial dysfunction; atherosclerosis; vascular remodeling; blood pressure; hypertrophy.**

HGF is a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells, and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis (1-3). Serum HGF concentration has been reported to be elevated in response to organ damage, such as in hepatitis and nephritis (1,4-6). Recently, we have reported that HGF is a novel member of endothelium-specific growth factors whose mitogenic activity is the most potent among various growth factors in human aortic endothelial cells (7). Moreover, the presence of a local HGF system has been described in various tissues including blood vessels (1-3,8). Therefore, the local HGF system may have a protective role in the regulation of local function. The local HGF system has been reported to be controlled by the balance of transforming growth factor (TGF-$\beta$), angiotensin II and HGF itself, since TGF-$\beta$ and angiotensin II are strong suppressers and HGF itself is a positive regulator of local HGF production (9-13).

On the other hand, endothelial dysfunction in hypertensive patients is well known (14-17). Probably, the loss of endothelium-derived substances (prostaglandin $I_2$, nitric oxide, C-type natriuretic peptide) may be related to the development and progression of atherosclerosis/arteriosclerosis in hypertensive patients (18,19). Taken together, we hypothesized that HGF might contribute to protection or repair of vascular endothelial cells. If so, serum HGF level might be elevated in response to endothelial cell damage induced by hypertension. Indeed, we have previously reported that serum HGF concentration in hypertensive patients was significantly higher than that in normotensive patients (20). However, the previous study failed to show the role of circulating HGF in hypertension, and the number of hypertensive patients in the previous study was limited. In this study, we evaluated the relationship between circulating HGF and the severity of blood pressure (BP) in normotensive individuals and hypertensive patients, to elucidate the role of HGF.

## MATERIALS AND METHODS

*Normotensive and hypertensive subjects.* For the study of serum HGF concentration, 57 normotensive controls (31 males and 26 fe-

0006-291X/98 $25.00

males, 59 ± 2 [mean ± S.E.] years old) and 171 age-matched hypertensive patients (91 males and 80 females, 61± 2 years old) were studied. There was no significant change in body mass index and renal function between normotensive and hypertensive subjects. The 171 hypertensive patients were divided into 69 hypertensive patients never treated (37 males and 32 females, 62 ± 2 years old) and 102 hypertensive patients treated with antihypertensive drugs for at least six months (54 males and 48 females, 60 ± 1 years old). Furthermore, untreated hypertensive patients were divided into 40 patients without complication (WHO I) and 29 patients with complications (WHO II + III). Alternatively, the normotensive group and hypertensive group were also divided into three stages of hypertension according to the guideline of WHO/ISH 1993, for further analysis (21). Secondary causes of hypertension were ruled out by clinical and laboratory findings. Patients with hepatic disease, lung disease, diabetes mellitus or hyperlipidemia were excluded from this study.

*Blood pressure measurement.* Blood pressure was measured in a standardized setting in the clinic in the morning before the subjects had taken any drugs, between 0830 and 1030 h. Blood pressure was taken with the patients lying, using a standard sphygmomanometer, to the nearest 2 mmHg, in the right arm, Korotkoff phases I and V being taken as systolic blood pressure (BP) and diastolic blood pressure, respectively. BP measurement was repeated at least three times in a blind fashion, and mean values of repeated measurements represented values of BP.

*Blood sampling and HGF measurement.* Antecubital venous blood was taken during the morning hours, 0700-0900, after an overnight fast. Serum was immediately separated by centrifugation at 4 °C, and stored at −20 °C until assay. Serum HGF concentration was assayed using a recently developed EIA for use in humans (20,22). Rabbit anti-human HGF IgG was coated on a 96-well plate (Corning) at 4°C for 15 hours. After blocking with 3% bovine serum albumin in phosphate-buffered saline (PBS), serum was added to each well, and the preparation was incubated for 2 hours at 25°C. Wells were washed three times with PBS containing 0.025% Tween 20 (PBS-Tween), then biotinylated rabbit anti-human HGF IgG was added and the preparation was incubated for 2 hours at 25°C. After washing with PBS-Tween, wells were incubated with horseradish peroxidase-conjugated streptoavidin-biotin complex in PBS-Tween. The enzyme reaction was initiated by adding substrate solution composed of 2.5 mg/ml o-phenylenediamine, 100 mM sodium phosphate, 50 mM citric acid, and 0.015% $H_2O_2$. The enzyme reaction was halted by adding 1 M $H_2SO_4$, and absorbance at 490 nm was measured.

*Statistical analysis.* All values are expressed as mean ± SEM. Analysis of variance with subsequent Bonferroni's test was employed to determine the significance of differences in multiple comparisons. Multiple regression analyses were used to assess the relation between blood pressure and other parameters. Values of $p<0.05$ were considered statistically significant.

## RESULTS

### *Serum HGF Concentration in Normotensive Subjects and Untreated Hypertensive Patients*

First, we measured serum HGF concentration in normotensive subjects and hypertensive patients who had never been treated with antihypertensive drugs. As shown in Fig. 1a, serum HGF concentration in untreated hypertensive patients was significantly higher than that in the normotensive group ($p<0.001$). To further analyze the relationship between serum HGF concentration and BP, we divided the hypertensive group into three stages of hypertension, according to WHO/ISH 1993. Serum HGF concentration in mild hypertensives (140-180 mmHg/90-105 mmHg; n = 28) was significantly higher than that in the normotensive group ($p<0.01$). Serum HGF concentration in moderate and severe hypertensives (over 180 mmHg/ over 105 mmHg; n = 12) was significantly higher than that in normotensive subjects and mild hypertensive patients ($p<0.001$, Fig. 1b). Moreover, serum HGF concentration in the hypertensive group without complication was significantly positively correlated with systolic BP ($r = 0.59$, $p<0.001$) and diastolic BP ($r = 0.51$, $p<0.001$) (Fig. 2).

Next, we evaluated the effect of complications on serum HGF concentration in hypertensive subjects.

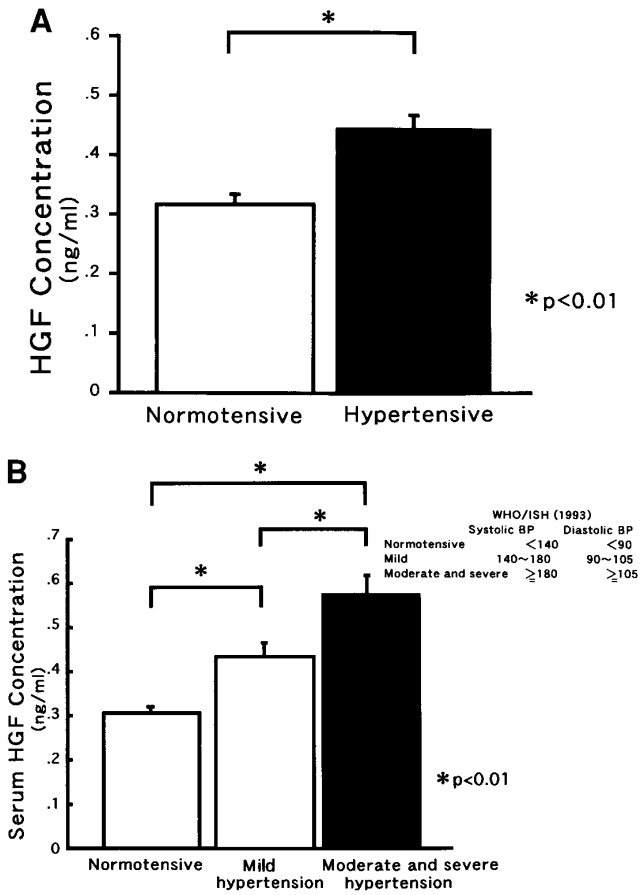


**FIG. 1.** (a) Serum HGF concentration in normotensive and hypertensive subjects. Normotensive = normotensive subjects (n = 57), Hypertensive = hypertensive subjects without complication (n = 40). **p<0.01, *p<0.05 vs. Normotensive. (b) Serum HGF concentration according to the WHO/ISH (1003) classification in normotensive and hypertensive subjects. Normotensive = normotensive subjects (n = 57), Mild hypertension = hypertensive subjects without complication (Systolic BP 140-180/Diastolic BP 90-105; n = 28), Moderate and severe hypertension = hypertensive subjects without complication (Systolic BP >180/Diastolic BP >105; n = 12). **p<0.01, *p<0.05 vs. Normotensive.

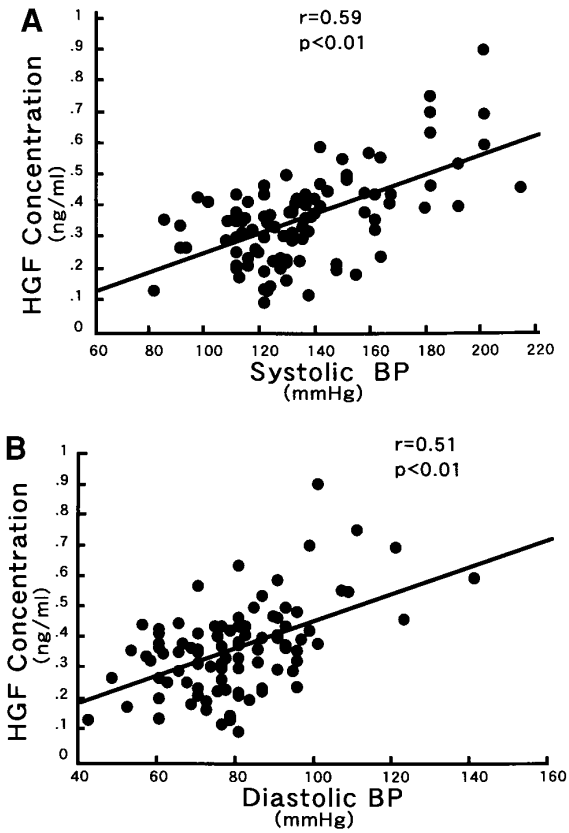


**FIG. 2.** Relation between serum HGF concentration and (a) systolic BP, and (b) diastolic BP. n = 97.

Therefore, we divided the hypertensive patients into two groups (WHO I and WHO II/III). Of importance, serum HGF concentration in hypertensive patients with complications (WHO II/III) was significantly higher than that in patients without complication (WHO I) ($p<0.001$, Fig. 3). To exclude the effect of BP, we compared serum HGF concentration in hypertensive subjects who showed the same BP level. Serum HGF concentration in hypertensive patients with complications was higher than that in hypertensive patients without complication in both mild, and moderate and severe hypertension ($p<0.01$, Fig. 4).

### *Serum HGF Concentration in Hypertensive Patients Treated with Antihypertensive Drugs*

Finally, to examine whether increased serum HGF concentration is due to increased BP and the presence of complications, we also measured serum HGF concentration in hypertensive patients treated with antihypertensive drugs. Interestingly, serum HGF concentration in hypertensive patients treated with antihypertensive drugs was significantly lower than that in hypertensive patients who had never been treated ($p<0.01$, Fig. 5a). There was no significant difference in

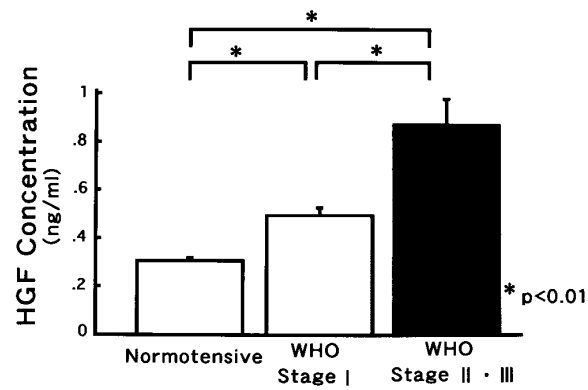


**FIG. 3.** Serum HGF concentration according to WHO stage in normotensive and hypertensive subjects. Normotensive = normotensive subjects (n = 57), WHO stage I = hypertensive subjects with WHO stage I (n = 40), WHO stage II/III = hypertensive subjects at WHO stage II and III (n = 29). **$p<0.01$, *$p<0.05$ vs. Normotensive.

serum HGF concentration between the normotensive subjects and hypertensive patients without complication treated with antihypertensive drugs (Fig. 5a). The effects of various antihypertensive drugs on serum HGF concentration were also evaluated. However, there were no significant differences among the patients treated with calcium antagonists, ACE inhibitors and other drugs (Fig. 5b).

## DISCUSSION

We have focused on the pathophysiological role of endothelial cells, since locally synthesized compounds from endothelial cells and vascular smooth muscle cells (VSMC) have been postulated to control

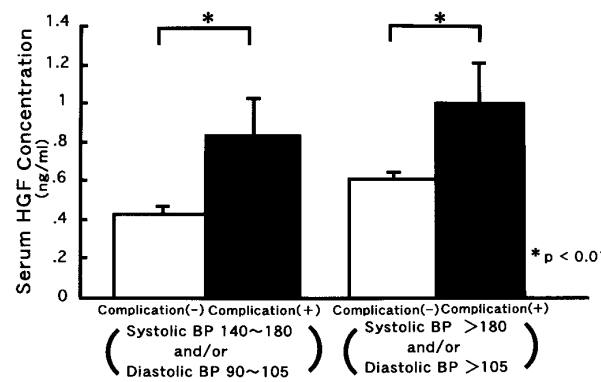


**FIG. 4.** Influence of complications on the serum HGF concentration in normotensive and hypertensive subjects. Complication (−) = subjects without complication, Complication (+) = subjects with complications. Systolic BP 140-180/Diastolic BP 90-105; Complication (−): n = 28, Complication (+): n = 24, Systolic BP >180/Diastolic BP >105; Complication (−): n = 12, Complication (+): n = 5. **$p<0.01$, *$p<0.05$ vs. Complication (−).

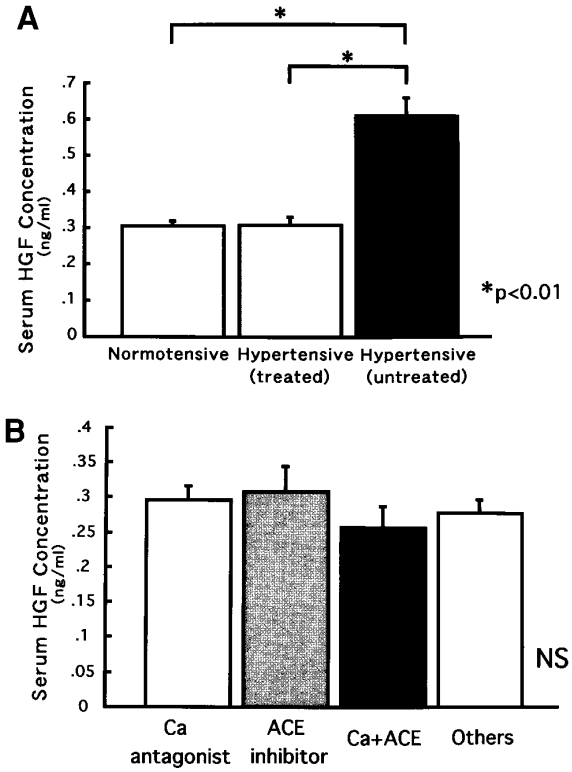


**FIG. 5.** (a) Serum HGF concentration in normotensive and hypertensive subjects with and without antihypertensive drug treatment. Normotensive = normotensive subjects (n = 57), Hypertensive (treated) = hypertensive subjects treated with antihypertensive drugs (n = 102), Hypertensive (untreated) = untreated hypertensive subjects (n = 69). **p<0.01, *p<0.05 vs. Complication (−). (b) Effects of different antihypertensive drugs on serum HGF concentration in hypertensive subjects. Ca antagonist = hypertensive subjects treated with calcium antagonists (n = 38), ACE inhibitor = hypertensive subjects treated with ACE inhibitors (n = 18), CA + ACE = hypertensive subjects treated with combination of calcium antagonists and ACE inhibitors (n = 19), Others = hypertensive subjects treated with other antihypertensive drugs except calcium antagonists and ACE inhibitors (n = 27). **p<0.01, *p<0.05 vs. Complication (−).

local vascular function (1-3,23-25). From this viewpoint, the maintenance of endothelial cells is very important, given their antiproliferative and vasodilating actions. Indeed, disruption or dysfunction of endothelial cells results in loss of multiple endothelium-derived substances ($PGI_2$, NO, CNP), resulting in the shift of balance of VSMC growth to abnormal growth such as in atherosclerosis. In hypertensive patients, the loss of vasodilating properties of resistance vessels is well known, probably due to the decrease in NO content and increase in hypertrophy and/or hyperplasia of VSMC induced by hypertension (14-17). Therefore, we sought an endothelium-specific growth factor that does not stimulate VSMC growth. As previously reported, HGF fulfills the above characteristics (13). HGF is also known to be a mesenchyme-derived pleiotropic factor which regulates cell growth, cell motility, and morphogenesis of various types of cells and thus is considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis (1-3). As mentioned earlier, a local HGF system (HGF and its receptor, c-met) has also been identified in vascular cells (8). Our preliminary data showed that vascular and cardiac HGF concentrations in spontaneously hypertensive rats (SHR) were significantly lower than those in Wistar-Kyoto rats (WKY) in response to increased local TGF-$\beta$ and angiotensin II, and serum HGF concentration was increased in SHR as compared to WKY (26).

As HGF may have a role in the prevention of endothelial dysfunction, we hypothesized that HGF might contribute to the protection or repair of vascular endothelial cells. If so, serum HGF level might be elevated in response to endothelial cell damage induced by hypertension. Our clinical data indicated that serum HGF concentration was significantly positively correlated with BP, consistent with our previous finding (20). In this study, we revealed a significant correlation of serum HGF concentration with systolic BP and diastolic BP, whereas our previous study demonstrated a significant correlation with only systolic BP. This difference may have been due to increased sample number, since our previous study showed a tendency, but no significance. The present data also indicated that serum HGF concentration was strongly correlated with systolic BP, rather than diastolic BP, since systolic BP has been thought to be related to the arteriosclerotic changes in the vasculature rather than diastolic BP. Of importance, the present study demonstrated a further elevation of serum HGF concentration in the hypertensive patients with complications such as arteriosclerosis. The level of serum HGF seems to be dependent on BP level as well as the presence of complications such as arteriosclerosis. Therefore, we examined whether elevation of serum HGF level in hypertensive patients is due to raised BP and the presence of complications. Interestingly, long-term treatment with antihypertensive drugs normalized serum HGF concentration to the same level as that in normotensive subjects. As it is well known that antihypertensive drugs such as ACE inhibitors and calcium antagonists improve endothelial function in hypertension (27-30), elevation of serum HGF concentration may be considered an index of arteriosclerotic vascular changes. Unfortunately, we failed to prove that the improvement of endothelial dysfunction would normalize serum HGF concentration, since there was no significant difference among various antihypertensive drugs. Our patients were mainly treated with

calcium antagonists, beta-blockers and ACE inhibitors, but not diuretics and vasodilators which are known to have minimal effects on the improvement of endothelial dysfunction. Further studies are necessary to determine the exact effect of endothelial dysfunction on serum HGF concentration.

What is the pathophysiological role of circulating HGF? Recent findings also revealed that HGF may play an important role in tissue regeneration (1-6). For example, HGF mRNA and blood HGF levels increase rapidly and markedly with hepatic injury and disease (4,31), and intravenously injected recombinant HGF markedly enhanced liver regeneration in vivo (32). Systemic HGF may work in tissue regeneration as a humoral mediator, in addition to autocrine-paracrine local HGF production. However, these results support the hypothesis, that systemic HGF is not sufficient to promote tissue regeneration due to decrease in local HGF production. Our preliminary results, that local HGF concentration in heart and blood vessels of experimental hypertensive rats was decreased as compared to normotensive control (26), are consistent with this hypothesis. Since serum HGF concentration is increased in hypertensive patients, serum HGF may act protectively against endothelial dysfunction in organs such as the vasculature and kidney.

Besides acting as a growth factor, HGF also has an important role in the regulation of thrombosis, as HGF belongs to the family of kringle proteins, characterized by a triple disulfide loop structure (kringles), that mediate protein/protein and protein/cell interactions (33). Although our previous study demonstrated no relationship between serum HGF concentration and total cholesterol, t-PA, PAI-I and Lp (a) (20), increased serum HGF concentration may have a role in the regulation of thrombosis. Overall, the present study demonstrated that serum concentration of HGF, a novel endothelial specific growth factor, is significantly elevated accompanied with the severity of hypertension, and that elevated serum HGF concentration could be normalized by anti-hypertensive treatment. These results suggested that HGF may be a new index of the severity of hypertension.

## ACKNOWLEDGMENTS

We thank Chihiro Noguchi for their excellent technical assistance. Dr. Ryuichi Morishita is the recipient of a Harry Goldblatt Award from the Council of High Blood Pressure, American Heart Association. Dr. Shin-ichiro Hayashi is a Research Fellow of the Japan Society for the Promotion of Science. This work was partially supported by the grants from the Japan Society for the Promotion of Science, the Osaka Kidney Foundation (OKF 96-0002) and ONO Medical Research Foundation.

## REFERENCES

1. Matsumoto, K., and Nakamura, T. (1993) *in* Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and C-Met Receptor. (Goldenberg, I. D., and Rosen, E. M., Eds.), pp. 225–249, Birkhauser Verlag, Basel.
2. Nakamura, T. (1991) *Prog. Growth Factor Res.* **3,** 67–86.
3. Boros, P., and Miller, C. M. (1995) *Lancet* **345,** 293–295.
4. Hamanoue, M., Takao, S., Shimazu, H., Noji, S., Matsumoto, K., and Nakamura, T. (1992) *Hepatology* **16,** 1485–1492.
5. Nagaike, M., Hirao, S., Tajima, H., Noji, S., Taniguchi, S., Matsumoto, K., and Nakamura, T. (1991) *J. Biol. Chem.* **266,** 22781–22784.
6. Igawa, T., Matsumoto, K., Kanda, S., Saito, Y., and Nakamura, T. (1993) *Am. J. Physiol.* **265,** F61–F69.
7. Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Aoki, M., Moriguchi, A., Yamada, K., Hayashi, S., Yo, Y., Nakano, H., Matsumoto, K., Nakamura, T., and Ogihara, T. (1996) *J. Hypertens.* **14,** 1067–1072.
8. Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Aoki, M., Moriguchi, A., Yamada, K., Hayashi, S., Yo, Y., Matsumoto, K., and Nakamura, T. (1995) *Biochem. Biophys. Res. Commun.* **215,** 483–488.
9. Matsumoto, K., Tashiro, K., Tajima, H., Okazaki, H., and Nakamura, T. (1992) *J. Biol. Chem.* **267,** 24917–24920.
10. Okajima, A., Miyazawa, K., and Kitamura, N. (1993) *Eur. J. Biochem.* **213,** 113–119.
11. Liu, Y., Michalopoulos, G., and Zarnegar, R. (1994) *J. Biol. Chem.* **269,** 4152–4160.
12. Gohda, E., Matsunaga, T., Kataoka, H., and Yamamoto, I. (1992) *Cell Biol. Int. Rep.* **16,** 917–926.
13. Nakamura, Y., Morishita, R., Higaki, J., Kida, I., Hayashi, S., Aoki, M., Yo, Y., Moriguchi, A., Matsumoto, K., Nakamura, T., and Ogihara, T. (1996) *J. Hypertens.* **14,** S70. (Abstr)
14. Cohn, J. M., and Finkelstein, S. M. (1992) *J. Hypertens.* **10,** 561–564.
15. Lockette, W., Otsuka, Y., and Carretero, O. A. (1986) *Hypertension* **8,** II-61–II-66.
16. Diedrich, D., Yang, Z., Buhler, F. R., and Luscher, T. F. (1988) *Am. J. Physiol.* **258,** H445–H451.
17. Panza, J. A., Quyyumi, A. A., Brush, J. E., and Epstein, S. E. (1990) *N. Engl. J. Med.* **323,** 22–27.
18. Dzau, V. J., and Gibbons, G. H. (1991) *Hypertension* **18,** III-115–III-121.
19. Krieger, J. E., and Dzau, V. J. (1991) *Hypertension* **18,** I-3–I-17.
20. Nakamura, Y., Morishita, R., Nakamura, S., Aoki, M., Moriguchi, A., Matsumoto, K., Nakamura, T., Higaki, J., and Ogihara, T. (1996) *Hypertension* **28,** 409–413.
21. 1993 Guidelines for the management of mild hypertension: Memorandum from WHO/ISH Meeting. ISH Hypertension News (Bulletin of the International Society of Hypertension, June) 1993; pp. 3–16.
22. Yamada, A., Matsumoto, K., Iwanari, H., Sekiguchi, K., Kawata, S., Matsuzawa, Y., and Nakamura, T. (1995) *Biomed. Res.* **16,** 105–114.
23. Tare, M., Parkington, H. C., Coleman, H. A., Neild, T. O., and Dusting, G. J. (1990) *Nature* **346,** 69–71.
24. Suga, S., Nakao, K., Itoh, H., Komatsu, Y., Ogawa, Y., Hama, N., and Imura, H. (1992) *J. Clin. Invest.* **90,** 1145–1149.
25. Dzau, V. J. (1993) *Curr. Opin. Nephrol. Hypertens.* **2,** 27–32.
26. Nakano, N., Moriguchi, A., Morishita, R., Kida, I., Tomita, N., Matsumoto, K., Nakamura, T., Higaki, J., and Ogihara, T. *Hypertension* (in press).
27. Thuillez, C., Mulder, P., Elfertak, L., Blaysat, G., Compagnon, P., Henry, J. P., Richard, V., Scalbert, E., and Desche, P. (1995) *Am. J. Hypertens.* **8,** 7S–12S.

28. Dohi, Y., Criscione, L., Pfeiffer, K., and Luscher, T. F. (1994) *J. Cardiovasc. Pharmacol.* **24,** 372–379.
29. Novosel, D., Lang, M. G., Noll, G., and Luscher, T. F. (1994) *J. Cardiovasc. Pharmacol.* **24,** 979–985.
30. Piana, R. N., Wang, S. Y., Friedman, M., and Sellke, F. W. (1996) *Circulation* **93,** 544–551.
31. Ito, T., Hayashi, N., Horimoto, M., Sasaki, Y., Tanaka, Y., Kaneko, A., Fusamoto, H., and Kamada, T. (1993) *Biochem. Biophys. Res. Commun.* **190,** 870–874.
32. Ishiki, Y., Ohnishi, H., Muto, Y., Matsumoto, K., and Nakamura, T. (1991) *Hepatology* **16,** 1227–1235.
33. Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K., and Shimizu, S. (1989) *Nature* **342,** 440–443.