THE EFFECT OF AGE ON ACTIVE AND CRYOACTIVATABLE INACTIVE PLASMA RENIN IN NORMAL SUBJECTS AND PATIENTS WITH ESSENTIAL HYPERTENSION

Mitsuaki Nakamaru, M.D., Toshio Oghara, M.D., Takeshi Hata, M.D.
Anna Maruyama, M.D., Hiroshi Mikami, M.D., Toru Naka, M.D.
Keiichi Iwanaga, and Yuichi Kumahara, M.D.

The effect of age on active and cryoactivatable inactive plasma renin levels was examined in 58 normal subjects and 58 patients with essential hypertension during recumbency and after stimulation with furosemide and ambulation. Active renin levels declined with age in both supine subjects and patients. Inactive renin levels did not change with age in normal subjects, while in hypertensive patients they decreased with age. Following stimulation with furosemide and ambulation, the levels of active renin increased but its responsiveness to the stimulus decreased with age in both groups. In contrast, inactive renin was not significantly influenced by furosemide administration and ambulation. These data show that an acute stimulation with furosemide and ambulation affects mainly the active form of plasma renin, and that the effect of age on inactive plasma renin in normal subjects may be different from that in patients with essential hypertension.

Human plasma, amniotic fluid, and kidney contain not only active but also inactive renin.\textsuperscript{1–9} Complete purification of inactive renin has not yet been accomplished and its biochemical nature and physiological significance are unclear. The mechanism of activation of renin and the relationship between inactive renin and high molecular weight renin, so called, big renin, remain to be elucidated. Inactive renin may be a precursor of the active form and may play an important role in the renin-angiotensin system.

Plasma renin activity (PRA) and its responsiveness to stimulation decrease with age.\textsuperscript{10–12} No data are available relating inactive renin to age. Examination of the inactive renin levels in plasma and its responsiveness to stimuli affecting renin release might help in clarifying the role of inactive renin in the pathogenesis of hypertension. For this purpose, we examined the effect of age on active and cryoactivatable inactive plasma renin before and after stimulation by furosemide and ambulation in normal subjects and patients with essential hypertension.

MATERIALS AND METHODS

Twenty-two young (15 to 35 years, 26.3 ± 1.3 (mean ± SEM) years), 17 middle-aged (36 to 60 years, 47.6 ± 2.0 years) and 19 elderly (62 to 84 years, 72.7 ± 1.7 years) normal subjects and 9 young (16 to 35 years, 26.8 ± 2.5 years), 17 middle-aged (38 to 59 years, 48.6 ± 1.4 years)

---

Key Words:
Age
Hypertension
Inactive renin
Cryoactivation

(Received January 6, 1981; accepted June 8, 1981)
Department of Medicine and Geriatrics, Osaka University Medical School, Fukushima-ku, Osaka 553, Japan
This work was supported by grants from the Ministry of Education and the Ministry of Health and Welfare, Japan.

Japanese Circulation Journal Vol. 45, November 1981 1231
and 32 elderly (61 to 81 years, 71.3 ± 1.2 years) patients with essential hypertension were studied. The proportion of men to women was almost equal in each group. All normal subjects had at least 3 casual blood pressures less than 140 mm Hg systolic and 80 mmHg diastolic. All hypertensive patients had at least 3 casual blood pressures greater than 160 mmHg systolic and 90 mm Hg diastolic. Patients with secondary hypertension or severe cardiovascular complications were excluded. Antihypertensive medications were discontinued at least 3 weeks prior to the study. Diets were unrestricted (sodium intake: 150–300 mEq/day). Informed consent was obtained from each subject. The study was approved by the Human Research Committee of the Department of Medicine and Geriatrics, Osaka University Medical School.

Blood samples for analyses were drawn from 8 to 10 a.m. after one hour of recumbency. Following collection of this sample, 40 mg of furosemide was injected intravenously and, after standing upright for one hour, blood sample was collected for measurement of active (PRA) and inactive plasma renin. Blood samples were collected in cold vacuum tubes containing disodium EDTA (1 mg/ml whole blood). Plasma was immediately separated in a refrigerated centrifuge and stored frozen at -20°C until assay.

PRA was measured by radioimmunoassay of the angiotensin I generated during incubation for 2 hours at pH 6.0 using a commercial kit. Total renin activity was measured by the cryoactivation method described by Sealey et al. Inactive renin was calculated as the difference between total renin activity after cryoactivation and PRA. All analyses were done within 2 weeks of sampling.

Statistical probability of differences was determined by Student's paired t-test. Regression lines and correlation coefficients were calculated by the method of least squares. Differences and correlation coefficients were considered statistically significant at p < 0.05.

**RESULTS**

There was a significant inverse correlation between supine PRA levels and age in both normal subjects (r = −0.59, p < 0.001) and hypertensive
Inactive Renin and Age

patients \((r = -0.35, \ p < 0.01)\) (Fig. 1). Supine inactive renin levels were not correlated with age in normal subjects, while there was a weak but statistically significant inverse correlation between supine inactive renin levels and age in hypertensive patients \((r = -0.27, \ p < 0.05)\) (Fig. 2).

Following stimulation with furosemide and ambulation, PRA levels increased significantly in all groups (Fig. 3). There was a significant inverse correlation between the furosemide-induced changes in PRA levels and age in both normal subjects \((r = -0.68, \ p < 0.001)\) and hypertensive patients \((r = -0.70, \ p < 0.001)\). In contrast, inactive renin levels did not respond significantly to stimulus in any of the groups (Fig. 4).

**DISCUSSION**

Inactive renin first reported by Lumbers\(^1\) can be activated \textit{in vitro} by acidification\(^1,3\) cold treatment\(^2,4\) or proteolytic enzymes such as pepsin\(^6\) cathepsin D\(^6\) trypsin\(^5,9\) or kallikrein\(^7,9\). However, the details of this activation mechanism are not clear. Atlas et al\(^14\) postulated that a common mechanism might be involved in cryo- and acid- activation. Recently it has been proposed that renal kallikrein, a serine protease, may be responsible for \textit{in vitro} activation of plasma renin\(^7,8\). Kallikrein is a potent activator of inactive renin \textit{in vitro}\(^2,8\). The storage site of kallikrein in the kidney is known to be close to the juxtaglomerular apparatus\(^15,16\). Sealey et al\(^9\) found a direct relationship between urinary kallikrein excretion and the proportion of active to total renin in plasma.

The physiological significance of inactive renin is obscure. We observed that active renin levels declined with age in supine normal subjects while cryoactivatable inactive renin levels did not change significantly. The decrease of active renin levels in the elderly might be caused by a decrease in the number of nephrons, a disturbance of renin secretion due to impairment of the juxtaglomerular apparatus, a decrease in sympathetic nerve activity and the suppression of renin secretion by increasing levels of blood pressure associated with age\(^11\). In the present study, active renin was decreased and inactive renin remained unchanged with age in normal subjects, resulting in a decrease of the proportion of active to total renin. Recently, we found that urinary kallikrein excretion decreased with age in normal subjects\(^17\). If renal kallikrein is involved in the in
vivo conversion of inactive renin as suggested by Sealey et al., a disturbance in this conversion might participate in the decrease in active renin with age in the normal subjects. Active renin levels in supine patients with essential hypertension and the responsiveness of active renin to stimulation also tend to diminish with age. In contrast with normal subjects, inactive renin levels decreased with age in hypertensive patients. These differences may be caused by underlying primary renal damage caused by long continued high blood pressure. Further studies will be needed to clarify this possibility.

Some investigators\textsuperscript{18,19} reported following furosemide administration, inactive renin increased only slightly, while there was a marked rise in active renin. Recently, Bailie et al.\textsuperscript{20} found that in the porcine kidney inactive renin increased in parallel with active renin after furosemide administration. Our observation that inactive renin remains unchanged after stimulation with furosemide and ambulation is in agreement with the report of Rumpf et al.\textsuperscript{21} Acute stimulation releases mainly active renin. In contrast, several investigators\textsuperscript{22,23} have shown that both chronic restriction of sodium intake and chronic diuretic therapy appreciably increase inactive renin. These observations suggest that inactive renin responds to salt depletion more slowly than does active renin.

In conclusion, an acute stimulation by furosemide administration and ambulation affected mainly the active form of plasma renin. Active renin was decreased with age in both normal subjects and patients with essential hypertension, but inactive renin was decreased with age only in hypertensive patients.

Acknowledgements
We are indebted to Dr. C.A. Nugent and Miss T. Kawano for their help.

REFERENCES
10. TUCK ML, WILLIAMS GH, CAIN JP, SULLIVAN JM, DLUHY RG: Relation of age, diastolic pressure and known duration of hypertension to presence of low renin essential hypertension. Am J Cardiol 32: 637, 1973
18. MILLAR JA, LECKIE BJ, SEMPLE PF, MORTON JJ, SONKODI S, ROBERTSON JIS: Active and


