PLASMA LEVEL OF NOREPINEPHRINE, CYCLIC AMP AND CYCLIC GMP IN ESSENTIAL HYPERTENSION

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Mean concentration of plasma norepinephrine of 66 normal subjects was 0.23 ± 0.02 ng/ml, but that of 81 hypertensives was 0.25 ± 0.02 ng/ml. There was no significant difference of plasma norepinephrine concentration between the normotensives and the hypertensives. However, a significant correlation was demonstrated between the concentration of norepinephrine and age in the normotensives (r = 0.51, p < 0.001). Nonetheless, this correlation was not found in the group with essential hypertension. Age matching revealed a significant higher concentration of norepinephrine in the twenties and the thirties and no significant difference in the forties and the fifties. There was also significant negative correlations between the concentration of norepinephrine and both the percent changes in mean blood pressure (r = −0.31, p < 0.01) and the percent changes in diastolic pressure (r = −0.32, p < 0.005) after twenty minutes in the recumbent position in the clinic. Mean concentration of plasma cyclic AMP and cyclic GMP of 70 normal subjects were 17.7 ± 0.4 pmol/ml and 4.3 ± 0.2 pmol/ml respectively. There was a significant correlation between the concentration of plasma cyclic GMP and age (r = 0.30, p < 0.001), and no correlation between the concentration of cyclic AMP and age in normal subjects. Mean concentration of plasma cyclic AMP of 68 hypertensives was 25.0 ± 0.8 pmol/ml and significantly higher than normal subjects (p < 0.001). But, that of cyclic GMP of the same patients was 4.4 ± 0.2 pmol/ml and there was no significant difference between the normotensives and the hypertensives.

Thus, it was suggested that sympathetic nervous activity was not elevated in older patients with essential hypertension but was elevated in younger patients and that the second messenger system of cyclic AMP was augmented in all patients with essential hypertension.

Key Words:
Norepinephrine
C-AMP
C-GMP
Essential hypertension

(Received June 16, 1980; accepted February 6, 1981)
The Second Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan
Presented in part at the 8th World Congress of Cardiology, September 17–23, 1978, Tokyo
Supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (No.157235, 1976)
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The relationship of catecholamines to essential hypertension is a subject which has been debated for many years. Recently, an accurate measurement of plasma catecholamine has been facilitated by the introduction of the radioenzymatic method, and there are a number of reports of increased levels of circulating cate-
cholamines in hypertensive patients. On the other hand, there have been a few report on the plasma concentration of cyclic nucleotides, which form the second messenger system of catecholamines in patients with essential hypertension.

Thus, we determined the concentration of plasma norepinephrine and that of plasma cyclic AMP and cyclic GMP in patients with essential hypertension in order to evaluate the autonomic nervous system activity in patients with essential hypertension.

MATERIALS AND METHODS

Sixty-six healthy normal subjects (45 males and 21 females, 14 to 68 years of age, mean age 42 ± 2 years old, mean systolic and diastolic blood pressure 119 ± 2/72 ± 1 mmHg) and 81 patients with essential hypertension (41 males and 40 females, 23 to 68 years of age, mean age 48 ± 1 years old, mean systolic and diastolic blood pressure 174 ± 3/101 ± 2 mmHg) were used for the measurement of the concentration of plasma norepinephrine. For the measurement of cyclic nucleotides, 70 healthy subjects (45 males and 25 females, 21 to 67 years of age, mean age 43 ± 2 years old) and 68 patients with essential hypertension (28 males and 40 females, 31 to 68 years of age, mean age 51 ± 1 years old, mean systolic and diastolic blood pressure 165 ± 2/101 ± 1 mmHg) were used. Patients and normal subjects were all studied in the clinic between 10:00 and 12:00 a.m. to minimize possible variations caused by diurnal changes. The average, casual blood pressure in recumbency in all the out-patients who visited more than three times was greater than 160/96 mmHg. All patients with essential hypertension were considered to be in stage I or stage II of the WHO classification and received no medication for at least 2 weeks before study. Patients with borderline hypertension were excluded from this study. After 20 minutes in the recumbent position blood pressure and pulse rate were recorded again and blood was taken from the antecubital vein and put into a tube containing either heparin or EDTA, and the plasma was rapidly separated from the cells by low speed centrifugation. According to Lake et al., sample drawn after 20 minutes in the recumbent position sufficiently reflects a basal state, since no differences in plasma norepinephrine concentrations were found between the samples after 20 minutes and 3 hours from subjects who kept recumbent. The plasma was then stored at −20°C for the assay.

A sensitive radioenzymatic method, developed by Henry et al. and modified by Lake et al., was used for the determination of the concentration of plasma norepinephrine. Following the extraction from plasma, norepinephrine was converted to 3H-epinephrine in the presence of phenylethya-
nolamine-N-methyl transferase and $^{3}$H-S-adenosylmethionine (New England Nuclear). A sensitive radioimmunoassay by Cailia et al.9.10 and Honma et al.11 was used for the determination of plasma cyclic AMP and cyclic GMP. Cyclic nucleotides in a 100 µl aliquot of plasma were directly succinized without prior deproteinization and then were bound to the antibody in an imidazole buffer. The recovery of cyclic AMP was 105 ± 5.5% and that of cyclic GMP was 93.0 ± 2.0%.

All values were expressed as mean ± standard error of mean (SEM). Student's $t$ test was used for the statistical analysis. Regression lines were fitted by the method of least squares. The $p$ values of less than 0.05 were considered significant.

RESULTS

Plasma norepinephrine and essential hypertension

Mean concentration of plasma norepinephrine in 66 normal subjects was 0.23 ± 0.02 ng/ml. There was a positive, significant correlation between the concentration of norepinephrine and age, and the concentration of plasma norepinephrine (Y) increased with age (X) in normal subjects ($Y = 0.0048X + 0.04$ ng/ml, $r = 0.51$, $p < 0.001$). Mean concentration of plasma norepinephrine of 81 hypertensives was 0.25 ± 0.02 ng/ml. There was no significant difference in norepinephrine concentration between the normotensives and hypertensives.

The comparative data for plasma concentration of norepinephrine in the normotensives and the hypertensives in each age bracket were shown in Fig. 1. Plasma concentration of norepinephrine of the normotensives in the twenties was 0.15 ± 0.02 ng/ml and that in the thirties were 0.13 ± 0.01 ng/ml. However, those of the hypertensives in the twenties and thirties were 0.31 ± 0.04 ng/ml and 0.20 ± 0.05 ng/ml respectively. Age matching revealed significant differences in plasma concentrations of norepinephrine in the 20- and 30-year-old age bracket ($p < 0.005$ and $p < 0.02$ respectively). There was no significant differences in 40- and 50-year-old age brackets. But in the sixties, it was 0.31 ± 0.04 ng/ml in the normotensives and 0.21 ± 0.03 ng/ml in the hypertensives. There was a significant difference between them ($p < 0.02$). However, significant correlation between the concentration of norepinephrine and age was not encountered in the group with essential hypertension. There was no significant correlation between plasma concentration of norepinephrine and systolic pressure ($r = +0.12$, $0.2 < p < 0.3$), and diastolic pressure ($r = +0.035$, $0.7 < p < 0.8$) in patients with essential hypertension after 20 minutes rest.

Figure 2 shows the relationship between the concentration of plasma norepinephrine and the percent changes in diastolic blood pressure after 20 minutes rest in patients with essential hypertension. There was significant negative correlation between the concentration of norepinephrine (Y) and the percent changes in diastolic
pressure (X) \( Y = -0.0042X + 0.225 \text{ ng/ml, } r = -0.32, p < 0.005 \). There were also significant negative correlations between the concentration of norepinephrine and changes in diastolic blood pressure \( (r = -0.34, p < 0.005) \), the percent changes in mean blood pressure \( (r = -0.31, p < 0.01) \) and changes in mean pressure \( (r = -0.34, p < 0.005) \). There were no significant correlation between the concentration of plasma norepinephrine and both changes in systolic pressure \( (r = -0.20) \) and the percent changes in systolic pressure \( (r = -0.12) \).

**Plasma cyclic nucleotides and essential hypertension**

Figure 3 shows the distribution pattern of the concentration of cyclic nucleotides in 70 normal subjects in each decade from the twenties to the seventies. Although the concentration of cyclic AMP was four times greater than that of cyclic GMP, the distribution pattern of cyclic GMP was similar. The mean concentration of cyclic AMP in normal subjects was 17.7 ± 0.4 pmol/ml and that of cyclic GMP was 4.3 ± 0.2 pmol/ml. Mean concentration of cyclic AMP in the sixties was 20.9 ± 1.1 pmol/ml and that of cyclic GMP was 5.3 ± 0.3 pmol/ml. Both levels were significantly elevated in relation to the other decades. There was a significant correlation between the concentration of plasma cyclic GMP and age in normal subjects \( (r = 0.30, p < 0.02) \), but no significant correlation between the concentration of cyclic AMP and age in normal subjects. The mean cyclic AMP concentration in the all hypertensives was 25.0 ± 0.8 pmol/ml and significantly elevated compared with that of the all normotensives \( (p < 0.001) \). However, no significant differences in cyclic GMP were found between the all hypertensives and the all normotensives.

Figure 4 compares the plasma concentration of cyclic AMP in normal subjects and patients with essential hypertension. Mean plasma concentration of cyclic AMP was 25.0 ± 2.5 pmol/ml in thirties, 23.9 ± 1.9 pmol/ml in the forties, 25.0 ± 1.2 pmol/ml in the fifties, and 26.2 ± 1.2 pmol/ml in the sixties in patients with essential hypertension. Thus, all showed significantly higher levels than the normal subjects. No significant correlation was demonstrated between plasma concentration of cyclic AMP and each blood pressure in patients with essential hypertension.

Figure 5 compares the plasma concentration of cyclic GMP in normal subjects and patients with essential hypertension. There was no statistically significant difference in cyclic GMP concentration in normal subjects and patients with essential hypertension in any decade. No significant correlations were demonstrated between cyclic GMP concentration and age or
blood pressure in patients with essential hypertension.

**DISCUSSION**

The mosaic theory of Page\textsuperscript{12} revealed that many factors are involved in the mechanisms of hypertension. Since the 1950s the hypothesis that the sympathetic nervous system could play a role in the etiology or in the maintenance of hypertension has been considered by several investigators. The investigation of the sympathetic nervous system has been based mainly on
the study of the metabolism of norepinephrine which constitutes the major neurotransmitter in peripheral postganglionic sympathetic nerve fibers. In human studies, the measurement of urinary catecholamines and metabolites has been most commonly used as a means of evaluation of the sympathetic nervous system. However, the results of the urinary excretion of norepinephrine in essential hypertensive subjects has not convincingly shown any regular increase indicating increased sympathetic nervous activity. When plasma catecholamine in patients with essential hypertension were first measured, the only abnormality noted in the plasma was that it occasionally appeared more in those patients than in comparable normal subjects after the infusion13. With the double isotope method, however, definitely increased plasma catecholamines were measured under standard conditions in essential hypertension in most laboratories, although there was some overlapping with normal values; about one-third of the values in the hypertensives were over the upper limit of the normal.4 On the other hand, in 1977, Lake and associates15 pointed out a correlation between the concentration of norepinephrine and age in normal population, and they showed that there was no difference of plasma level of norepinephrine between controls and patients with essential hypertension. Our findings in the present study also showed a significant difference in plasma concentration of norepinephrine between normal subjects and patients with essential hypertension in younger patients. Sever and associates16 reported a linear relationship between age and plasma norepinephrine in the normotensives, a relationship which did not occur in the hypertensives and is thought to be attributable to the raised plasma norepinephrine levels in many younger patients. These observations were completely consistent with our results. On the other hand, in our study of cyclic nucleotides, we demonstrated a significant correlation between the concentration of plasma cyclic GMP and age and no correlation between the concentration of cyclic AMP and age in normal subjects as well as a significant elevation of basal plasma concentration of cyclic AMP and no elevation of cyclic GMP in patients with essential hypertension in all age-matched study. There was no parallel relationship between the concentration of norepinephrine and that of cyclic AMP in age matched patients with essential hypertension. This discrepancy may be attributable to a subgroup of some patients with essential hyper-

tension who would have a hormonal hyperactivity other than norepinephrine or who would have a supersensitivity of the receptor site in sympathetic nervous system.

In conclusion, it was suggested that sympathetic nervous activity was not elevated in older patients with essential hypertension but was elevated in younger patients with essential hypertension, and it was considered that the second messenger system of cyclic AMP was augmented in all patients with essential hypertension.

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