Plasma Levels of Cyclic Nucleotides in Patients with Essential Hypertension

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Summary
We studied plasma levels of cyclic nucleotides and their responses to submaximal exercise as an endogenous adrenergic stimulation in normal subjects, untreated and treated patients with essential hypertension to assess the roles of various hormones and the autonomic nervous system in essential hypertension.

Plasma c-AMP level was significantly higher in untreated, diuretic-treated patients and those treated with propranolol than in normal subjects, but plasma c-GMP level was comparable in normal subjects and untreated patients. Plasma c-AMP decreased significantly, whereas plasma c-GMP increased significantly, after chronic propranolol therapy. Plasma c-AMP increased significantly after submaximal exercise in normal subjects, untreated patients and those treated with propranolol, but plasma c-GMP increased significantly only in normal subjects. The increase in plasma c-AMP was significantly higher in untreated patients than in normal subjects and patients treated with propranolol. Moreover, the percent increase in plasma c-AMP was significantly higher in untreated patients than in those treated with propranolol.

Therefore, it is suggested that the sympathetic nervous system may be hyperactive, and that a hyperreactivity of the β-adrenergic receptors may play an important role in essential hypertension.

Additional Indexing Words:
c-AMP c-GMP Diuretics β-adrenergic blockade
Submaximal exercise β-adrenergic receptor Hyperreactivity

The mechanistic concept of hypertension is not new, and it was suggested that hypertension might be considered a disease of dis-regulation.1) The importance of hormones and neurotransmitters in the regulation of blood pressure and their involvement in hypertension are well established.

C-AMP was initially discovered in the late 1950's as the intracellular mediator of the glycogenolytic effect of epinephrine and glucagon in the liver, and it has since come to be recognized as a second messenger mediating...
a variety of hormones.\textsuperscript{21} C-GMP was found in 1963 by Ashman et al\textsuperscript{3} and has been known to be increased by acetylcholine\textsuperscript{4} and \(\alpha\)-adrenergic agents.\textsuperscript{5} It is apparent that some of the nucleotides escape from their cells of origin into the extracellular fluids, including plasma, urine and cerebrospinal fluid. Plasma levels of cyclic nucleotides were measured in this study, because it was suggested that the concentration of free, metabolically active c-AMP was only a small fraction of the total tissue c-AMP.\textsuperscript{6,7} The changes in free, functionally active cyclic nucleotides may be understood by measuring plasma levels rather than tissue levels. Therefore, plasma cyclic nucleotides may partially reflect a hormonal and/or neurogenic component in essential hypertension.

This study is composed of 2 parts. In Part I, plasma levels of cyclic nucleotides were measured in normal subjects, untreated and treated patients with essential hypertension. In Part II, a multi-stage treadmill test of submaximal exercise was performed to evaluate the responses of plasma cyclic nucleotides to endogenous adrenergic stimulation.

**MATERIALS AND METHODS**

This study is composed of 2 parts.

**Part I:** The subjects were divided into the following 5 groups.

1) Normal subjects: 70 healthy subjects who were considered normal after a complete medical check-up were included in this group (45 men and 25 women, 21 to 67 years of age, mean age 43.2 ± 1.6).

2) Untreated patients: 68 patients with essential hypertension who were treated with no antihypertensive drugs for at least 2 weeks (28 men and 40 women, 31 to 68 years of age, mean age 50.5 ± 1.2).

3) Diuretic-treated patients: 41 patients with essential hypertension who were treated with diuretics (thiazides and potassium-sparing diuretics) for more than 1 week (14 men and 27 women, 33 to 69 years of age, mean age 51.9 ± 1.4).

4) Patients treated with propranolol: 26 patients with essential hypertension who were treated with propranolol, 30 to 60 mg/day, alone (n=3) or in combination with diuretics (n=23) for more than 3 weeks (5 men and 21 women, 33 to 68 years of age, mean age 50.9 ± 1.8).

5) Follow-up group: 8 patients with essential hypertension who were treated with no antihypertensive drugs for at least 2 weeks (2 men and 6 women, 41 to 62 years of age, mean age 47.0 ± 2.5). They were all re-examined after propranolol therapy for more than 3 weeks. One of 8 patients was treated only with propranolol, 60 mg/day, and others were given propranolol in combination with diuretics.

**Part II:** The subjects were divided into the following 3 groups, and each of them carried out a multi-stage treadmill test of submaximal exercise.

1) Normal subjects: 7 normotensive volunteers who were apparently healthy were included in this group (6 men and 1 woman, 24 to 59 years of age, mean age 35.9 ± 4.1).
2) Untreated patients: 8 patients with essential hypertension who were treated with no antihypertensive drugs for at least 2 weeks (4 men and 4 women, 30 to 59 years of age, mean age 41.1±3.6).

3) Patients treated with propranolol: 7 patients with essential hypertension who were treated with propranolol, 30 to 60 mg/day, in combination with diuretics for more than 3 weeks (2 men and 5 women, 25 to 59 years of age, mean age 45.6±4.4).

All patients in this study were treated with no other antihypertensive drugs and given no other agents which were considered to exert an effect on the metabolism of cyclic nucleotides. The average, casual blood pressure in recumbency during outpatient visits for more than 2 times was greater than 160/95 mmHg in all patients. Each patient was given a sufficient work-up to exclude any known cause of hypertension. This evaluation included electrocardiography (ECG), cardio-pulmonary roentgenography, urinalysis, serum creatinine, electrolytes and the like. Furthermore, on an elective basis, ocular fundus, drip-infusion pyelography and endocrinological examinations such as plasma aldosterone, catecholamines and cortisol were included. All patients were considered to be mild or moderate hypertensives and to correspond to Stage I or II of the WHO classification (1962).

Blood samples were taken by venipuncture from a vein in the forearm, as follows. In normal subjects of Part I, blood samples were obtained in the sitting position, but in Part I patients, blood pressure and heart rate were measured in the supine position after a 10 to 20 min resting period, and then blood samples were taken. In Part II, after a 20 min resting period in the supine position, each subject performed a standing test on the treadmill at 10% grade for 10 min and then carried out a multi-stage treadmill test of submaximal exercise according to Bruce's protocol. The treadmill test began with walking slowly for 3 min at 1.7 mph at 10% grade (Stage I), and then speed and grade increased every 3 min with 3 min of intervening resting periods up to the submaximal endpoint or, if possible, Stage V. A target heart rate was determined as 85% of the predicted maximal heart rate for age. Blood samples were initially obtained in the supine position after a 20 min resting period, subsequently in the upright position after a 10 min standing period, and in the supine position immediately after the treadmill exercise. During blood sampling, right brachial blood pressure was measured by the standard auscultatory method. Heart rate and a bipolar ECG with a positive electrode in the V5 position were monitored using an Avionics Stress Test Monitor throughout the exercise test.

Mean blood pressure was calculated as diastolic pressure plus 1/3 of pulse pressure and pressure rate product (PRP) as heart rate multiplied by systolic blood pressure.

Blood (1 to 2 ml) was collected into a chilled tube packed in ice with 20 µl of 0.5 M EDTA and immediately centrifugated at 4°C. Plasma was separated and frozen at −20°C until assayed (within a few months of collection).

Cyclic nucleotides were simultaneously measured in duplicate by the radioimmunoassay method of Honma et al. by use of commercially available kits (Yamasa Cyclic AMP Assay Kit & Cyclic GMP Assay Kit, Yamasa Shoyu Co, Ltd). In this assay, cyclic nucleotides in a 100-µl aliquot of plasma were directly succinated without a prior deproteinization and then were bound to the antibody in an imidazole buffer. The recovery of c-AMP was 105±5.5% and that of c-GMP was
93±2.0%.

All values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed with the Student's t-test for paired and unpaired data. Regression lines were fitted by the method of least squares.

Results

Part I:

Fig. 1 shows the age-matched distribution pattern of plasma levels of cyclic nucleotides in normal subjects. The mean c-AMP level of overall subjects was 17.7±0.4 pmol/ml. As our co-worker had reported,10) the highest c-AMP level was in the sixties (20.7±1.1 pmol/ml) and the lowest level was in the forties (16.3±0.9 pmol/ml). There was a significant difference between subjects in the sixties and all subjects (p<0.025). The mean c-GMP level of all subjects was 4.3±0.2 pmol/ml. As with c-AMP, the highest level was in the sixties (5.3±0.3 pmol/ml), but the lowest level was in the twenties (3.7±0.3 pmol/ml). There was a significant difference between subjects in the sixties and overall subjects (p<0.025). Fig. 2 shows a weak correlation between plasma c-GMP level and age in normal subjects (r=0.296, p<0.02), but no significant correlation was found between plasma c-AMP level and age.

Table I shows the hemodynamic findings in untreated and treated patients with essential hypertension. Blood pressure was significantly higher in untreated patients than in diuretic-treated patients and those treated with

![Graph showing plasma cyclic nucleotide levels in normal subjects](image)
Fig. 2. Correlation between plasma c-GMP level and age in normal subjects.

Table I. Hemodynamic Findings in Patients with Essential Hypertension

<table>
<thead>
<tr>
<th></th>
<th>Untreated patients</th>
<th>Treated patients (diuretics)</th>
<th>Treated patients (propranolol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>165.1 ± 2.2</td>
<td>154.1 ± 3.3(^b)</td>
<td>153.2 ± 5.1(^a)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>101.1 ± 1.2</td>
<td>92.6 ± 1.9(^c)</td>
<td>92.7 ± 2.1(^c)</td>
</tr>
<tr>
<td>Mean</td>
<td>122.4 ± 1.3</td>
<td>113.1 ± 2.1(^c)</td>
<td>112.8 ± 2.8(^c)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75.5 ± 1.2</td>
<td>73.3 ± 2.1</td>
<td>65.6 ± 1.5(\text{cd})</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \(^a\) p<0.05, \(^b\) p<0.01, \(^c\) p<0.001 when compared with untreated patients. \(\text{d}\) p<0.01 when compared with treated patients (diuretics).

propranolol. Heart rate was significantly lower in patients treated with propranolol than in both untreated and diuretic-treated patients. As shown in Fig. 3, the mean plasma c-AMP levels of 54 normal subjects who were over the age of 30 (33 men and 21 women, 31 to 67 years of age, mean age 48.3±1.4), 68 untreated patients, 41 diuretic-treated patients and 26 patients treated with propranolol were 17.9±0.5, 25.0±0.8, 26.3±1.1, and 22.2±0.9 pmol/ml, respectively. There was a significant difference between normal subjects and untreated (p<0.001), diuretic-treated patients (p<0.001), those treated with propranolol (p<0.001). Moreover, there was a significant difference between untreated patients and those administered propranolol (p<0.05) and between diuretic-treated patients and those on propranolol (p<0.05). The mean plasma c-GMP levels of normal subjects, untreated, diuretic-treated patients and those given propranolol were 4.5±0.2, 4.4±0.2, 5.4±0.3, and 5.2±0.3 pmol/ml, respectively. There was a significant
Fig. 3. Plasma cyclic nucleotide levels in normal subjects and patients with essential hypertension. The number of subjects in each group is shown in columns. Values are mean±SEM.

difference between normal subjects and diuretic-treated patients (p<0.05), those treated with propranolol (p<0.05). There was also a significant difference between untreated and diuretic-treated patients (p<0.01), those treated with propranolol (p<0.05). No differences were found among the 4 groups in terms of age.
As shown in Fig. 4, the mean plasma c-AMP level was significantly higher in untreated and diuretic-treated patients than in normal subjects in each age bracket. The mean plasma c-AMP level was significantly higher in patients treated with propranolol than in normal subjects in their forties and fifties. Moreover, the mean plasma c-AMP level tended to be higher in the sixties (0.05<p<0.1). As shown in Fig. 5, there were no definite trends in the age-matched plasma c-GMP level. No significant correlation was demonstrated between the plasma c-AMP level and age, blood pressure or heart rate in untreated patients. Similarly, no significant correlation was demonstrated between the plasma c-GMP level and age, blood pressure or heart rate in untreated patients. As shown in Table II, after chronic pro-

![Graph](image)

**Fig. 5.** Age-matched plasma c-GMP levels in normal subjects and patients with essential hypertension. The number of subjects is shown in columns. Values are mean±SEM.

<table>
<thead>
<tr>
<th>Table II. Effects of Chronic Propranolol Therapy on Plasma Cyclic Nucleotide Levels and Hemodynamic Findings</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Before therapy</strong></td>
</tr>
<tr>
<td>c-AMP (pmol/ml)</td>
</tr>
<tr>
<td>c-GMP (pmol/ml)</td>
</tr>
<tr>
<td>BP S (mmHg)</td>
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<tr>
<td>D (mmHg)</td>
</tr>
<tr>
<td>M (mmHg)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
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<tr>
<td><strong>After therapy</strong></td>
</tr>
<tr>
<td>c-AMP (pmol/ml)</td>
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<td>c-GMP (pmol/ml)</td>
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<td>BP S (mmHg)</td>
</tr>
<tr>
<td>D (mmHg)</td>
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<tr>
<td>M (mmHg)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BP=blood pressure; S=systolic; D=diastolic; M=mean.

*p<0.05, **p<0.01 when compared with values before therapy.
pranolol therapy, plasma c-AMP decreased significantly \((p<0.01)\), but, conversely, plasma c-GMP increased significantly \((p<0.05)\). Blood pressure and heart rate decreased significantly. No significant correlation was demonstrated between the change in plasma cyclic nucleotides and the decrease in blood pressure or heart rate.

Part II:

There were no significant differences among the 3 groups in terms of age. The mean exercise durations of normal subjects, untreated patients and those treated with propranolol were \(13.9\pm0.4\), \(11.1\pm0.9\), and \(10.5\pm0.9\) min, respectively. There was a significant difference between normal subjects and untreated patients \((p<0.02)\), those treated with propranolol \((p<0.01)\). Fig. 6 shows the hemodynamic variables of 3 groups in the supine, standing position and at the submaximal endpoint. Mean systolic blood pressure at the submaximal endpoint was significantly higher in untreated patients than in normal subjects \((p<0.025)\). Mean heart rate at the submaximal endpoint was significantly higher in normal subjects \((p<0.01)\) and untreated patients \((p<0.01)\) than in those treated with propranolol. Mean PRP at the submaximal endpoint was significantly higher in untreated patients than in those treated with propranolol \((p<0.025)\).

As shown in Fig. 7, plasma c-AMP increased significantly after submaximal exercise in each group compared with that in the supine position, whereas plasma c-GMP increased only in normal subjects. Mean plasma

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![Graph](image-url)

**Fig. 6.** Hemodynamic responses to standing and submaximal exercise. Differences among 3 groups in the supine and standing position are not represented in this figure. Values are mean±SEM. BP = blood pressure; HR = heart rate; PRP = pressure rate product; Stand. = standing; Submax. = submaximal.
Fig. 7. Responses of plasma cyclic nucleotides to standing and submaximal exercise. Values are mean±SEM. Stand.=standing; Submax.=submaximal.

Table III. Increases and Percent Increases in Plasma Cyclic Nucleotides Produced by Submaximal Exercise

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Untreated patients</th>
<th>Treated patients (propranolol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δc-AMP (pmol/ml)</td>
<td>6.6±0.9*</td>
<td>10.2±1.2</td>
<td>4.7±0.7**</td>
</tr>
<tr>
<td>%</td>
<td>37.0±5.7</td>
<td>42.7±4.7</td>
<td>23.3±3.6**</td>
</tr>
<tr>
<td>Δc-GMP (pmol/ml)</td>
<td>3.2±1.2</td>
<td>2.2±1.1</td>
<td>3.4±1.5</td>
</tr>
<tr>
<td>%</td>
<td>56.2±17.9</td>
<td>35.4±13.1</td>
<td>53.9±20.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
* p<0.05, ** p<0.01 when compared with untreated patients.

c-AMP level was significantly higher in untreated patients than in normal subjects in the supine (p<0.02) and standing position (p<0.025). As shown in Table III, the increase in plasma c-AMP (Δc-AMP) produced by submaximal exercise was significantly higher in untreated patients than in normal subjects (p<0.05) and patients treated with propranolol (p<0.01). The percent increase in plasma c-AMP was significantly higher in untreated patients than in those treated with propranolol (p<0.01). But there were no differences among the 3 groups in the increase (Δc-GMP) or the percent increase in plasma c-GMP. No significant correlation was found between the increase in each plasma cyclic nucleotide and the increase in systolic blood pressure, heart rate or PRP in all subjects. And there was no significant correlation between the percent increase in each cyclic nucleotide and the percent increase in systolic blood pressure, heart rate or PRP in every subject.
DISCUSSION

There are several reports concerning the plasma levels of cyclic nucleotides in patients with essential hypertension. After 30 min of isoproterenol infusion, a significantly higher increase in plasma c-AMP and heart rate was seen in the hyperkinetic form of borderline essential hypertension than in control subjects, and propranolol abolished hemodynamic and humoral responses to a similar degree in both groups. It was suggested that these findings were compatible with a hyperreactivity of the β-adrenergic receptors or of the adenylate cyclase or both.\(^{11}\)

Many studies on plasma and urinary catecholamines in essential hypertension were reported. De Champlain et al\(^{12}\) and DeQuattro et al\(^{13}\) showed increased concentrations of plasma catecholamines in essential hypertension. Franco-Morselli et al\(^{14}\) also showed increased plasma epinephrine concentration in benign essential hypertension. However, Lake et al\(^{15}\) and Sever et al\(^{16}\) found a linear relationship between plasma norepinephrine and age in normotensives, and no difference in mean plasma norepinephrine was detected between hypertensives and normotensives.

There is general agreement that the diuretics reduce plasma and extracellular volumes during the first week or two of administration. From 1968, serial studies of hypertensive patients receiving only diuretics showed that chronic treatment was associated with a decrease in plasma volume by 7 to 10%, and a probable reduction of extracellular volume. This diuretic-induced fall in extracellular volume stimulates renin secretion, and a diuretic-induced reduction of peripheral resistance was also reported.\(^{17}-^{19}\) The precise mechanism of the antihypertensive effect of β-adrenergic blockades is unknown. Nevertheless, it does not seem to be one of the associated properties but rather a function of their β-adrenoceptor inhibitory action. In a group of patients already on a diuretic, propranolol was found to increase plasma volume almost to pre-diuretic control level, even though the addition of propranolol produced a further fall in blood pressure.\(^{20}\)

In this study, both cyclic nucleotides showed a remarkable resemblance in the age-matched distribution pattern in normal subjects, and there was also a positive correlation between plasma c-GMP level and age. In contrast to previous studies,\(^{11,21}\) plasma c-AMP level was significantly higher in untreated patients than in normal subjects, whereas plasma c-GMP level was comparable in both groups. Moreover, no significant correlation was found between plasma c-AMP or c-GMP level and age in untreated patients. The mean plasma levels of both cyclic nucleotides were significantly higher in diuretic-treated patients and those treated with propranolol than in normal
subjects. Mean plasma c-AMP level was 5.1% greater in diuretic-treated patients than in untreated ones. Similarly, mean c-GMP level was 22.7% greater in diuretic-treated patients than in untreated patients. This elevation of cyclic nucleotides may be due to a direct action of the diuretics or a decrease in plasma volume. Diuretic-induced renin secretion may result in this elevation of cyclic nucleotides, especially of c-GMP, in the light of the suggestion by Rosman et al\textsuperscript{22} that c-GMP mediated the pressor effect of angiotensin as well as of $\alpha$-adrenergic agents. Plasma c-AMP, blood pressure and heart rate decreased significantly after chronic treatment with relatively small doses of propranolol, whereas plasma c-GMP increased significantly. Since an increase in plasma concentrations of catecholamines was observed following treatment with propranolol,\textsuperscript{23} the decrease in plasma c-AMP probably resulted from the $\beta$-adrenoceptor inhibitory action of propranolol, and the increase in plasma c-GMP was partially attributed to the increase in plasma concentrations of catecholamines. However, it was difficult to account for the increase in plasma c-GMP precisely because the majority of this follow-up group were treated in combination with diuretics.

Many physiological maneuvers have been used to evaluate the role of the autonomic nervous system, including static and dynamic exercise. Profound humoral changes have been reported during treadmill exercise, such as elevated norepinephrine, epinephrine, glucagon, growth hormone, and c-AMP.\textsuperscript{24}–\textsuperscript{26} An increase in plasma norepinephrine concentration during recumbency and exercise was described following treatment with propranolol.\textsuperscript{23} Similarly, it was found that the cardioselective $\beta$-adrenergic blockade, atenolol, produced a significant increase in plasma norepinephrine both in recumbency and during exercise, and the ratio of plasma norepinephrine concentration during exercise to the base line concentration did not change significantly.\textsuperscript{27}

In the present study, although the exercise duration was slightly longer in normal subjects, the increase in plasma c-AMP produced by submaximal exercise was much more pronounced in untreated patients than in normal subjects. The increase and the percent increase in plasma c-AMP were also more marked in untreated patients than in those treated with propranolol. It is well known that glucagon is a potent stimulus of the adenylate cyclase-cyclic AMP system, but $\beta$-adrenergic receptors do not mediate this c-AMP response to glucagon. This increase in plasma c-AMP was supposed to be mainly mediated by $\beta$-adrenergic receptors because it was certainly suppressed by chronic propranolol therapy. Therefore, findings suggest an increased responsiveness of the $\beta$-adrenergic receptors in patients with essential hypertension.
The principal finding of this study is that plasma c-AMP level is significantly higher in untreated patients with essential hypertension than in normal subjects, whereas plasma c-GMP level is comparable in both groups. In addition, plasma c-AMP decreases after chronic propranolol therapy, and the increase in plasma c-AMP in response to endogenous adrenergic stimulation is more pronounced in untreated patients with essential hypertension.

Although there is no conclusive evidence for the role of catecholamines in the development or maintenance of essential hypertension, our data may well suggest a state of hyperactivity of the sympathetic nervous system and reflect an abnormal responsiveness of the hormonal adrenergic receptors in the heart and other tissues rather than the direct action of increased concentrations of catecholamines. In a previous study, an increase of sympathetic tone and a decrease of parasympathetic tone were reported in borderline hypertension. Our data may confirm this report, but the role of the parasympathetic nervous system has not yet been sufficiently clarified.

In conclusion, our data suggest that the sympathetic nervous system may be hyperactive, and a hyperreactivity of the β-adrenergic receptors may play an important role in essential hypertension, as previously suggested in borderline essential hypertension. Further comparative studies will be needed to elucidate the balance of sympathetic and parasympathetic nervous activities important for the regulation of the blood pressure.

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