Plasma cyclic AMP responses to adrenaline administration in patients with spinocerebellar degeneration, bronchial asthma, and diabetes mellitus

—A Preliminary Report of A Clinical Test for Detecting $\beta$-adrenergic Dysfunctions—

Fumihiko Okada, MD, Tukasa Koyama*, MD, Hajime Ide**, MD, Miyuki Honma***, PhD and Michio Ui***, PhD

Plasma cyclic AMP responses to adrenaline administration in normal volunteers, patients with spinocerebellar degeneration, bronchial asthma, pulmonary emphysema, and diabetes mellitus were studied. Intramuscular administration of low doses (0.1 – 0.4 mg/person) of adrenaline caused a dose-dependent increase in plasma cyclic AMP. The increase in cyclic AMP was completely prevented by propranolol, while it was not affected by phentolamine or atropine. In patients with spinocerebellar degeneration, the concentrations of plasma cyclic AMP both before and after adrenaline administration were lower than in normal subjects. In asthmatic patients, the plasma cyclic AMP increase after adrenaline administration was smaller than that of the healthy controls. The plasma concentration of cyclic AMP in patients with insulin-dependent diabetes reached the peak level more slowly than in diabetic patients with dietary control alone. Examining changes in the plasma cyclic AMP level after adrenaline administration appears to be a useful means for assessing the degree of $\beta$-adrenergic dysfunction.

Key Words: Spinocerebellar degeneration, Bronchial asthma, Pulmonary emphysema, Diabetes mellitus, Adrenaline, Plasma cyclic AMP

Adrenaline is well known to increase the plasma concentration of cyclic adenosine 3',5'-monophosphate (cyclic AMP) in humans1-4. Although the origin of plasma cyclic AMP is not clear, catecholamine-stimulated increase of plasma cyclic AMP may reflect $\beta$-adrenergic functions, since it is prevented by simultaneous administration of propranolol1.

The present preliminary report will show that the plasma cyclic AMP response to a single injection of low doses of adrenaline exhibits some anomalies in patients whose $\beta$-adrenergic functions may be affected.

SUBJECTS AND METHODS

Patients and subjects

Consent for the study was obtained from each patient and subject after full explanation of the purpose, nature, and risk of all procedures used. The healthy subjects were 25 normotensive volunteers (23 males and 2 females) in excellent physical condition between the ages of 19 and 59. The following 4 categories of patients were studied: (1) 10 patients with spinocerebellar degeneration (Marie’s cerebellar ataxia) (6 males, 4 females, age 33 to 57), before and one month from the Health Administration Center, Hokkaido University, Sapporo, Japan.

*Department of Psychiatry and Neurology, Hokkaido University School of Medicine, Sapporo, Japan.

**Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan.

***Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan.

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Reprint request to: Fumihiko Okada, MD, The Health Administration Center, Hokkaido University, N8, W5, Kita-ku, 060, Japan.

after treatment with a daily dose of 300 – 600 mg phthalazinol (a potent cyclic AMP phosphodiesterase inhibitor), (2) 9 patients with bronchial asthma, on the basis of airway resistance 0.5 – 6 years (6 males, 3 females, age 18 to 64), some on the daily maintenance therapy with bronchodilators such as the salbutamol aerosol, and three on monthly 40 mg triamcinolone intramuscular injections (all therapy was discontinued for at least 3 days before the studies), (3) 9 patients suffering from pulmonary emphysema for 2 – 10 years (all male, age 61 to 76), some on the daily maintenance therapy with bronchodilators such as the salbutamol aerosol (all therapy had discontinued for at least 3 days before the studies), and (4) 16 patients with diabetes mellitus with or without peripheral neuropathy, 8 cases (5 males, 3 females, age 35 to 64), with a dietary control alone, who had been diabetic for 1 – 9 years (the fasting blood sugar level; 84 – 114 mg/dl), and 8 cases (all male, age 23 to 50) with 3 – 16 years insulin-dependent diabetes (the fasting blood sugar level; 150 – 270 mg/dl). All of the insulin-dependent diabetic patients were receiving continued treatment with insulin (18 – 125 units/day) except for the morning insulin dose in the day of the study. No oral hypoglycemic medication was applied.

Test procedures

In the morning, 14-h fasted subjects were kept in a resting supine position for 60 min. The test was carried out from 9.00 to 11.00 a.m.; it has been reported that there is no circadian variation in plasma cyclic AMP concentrations from 8.00 to 20.00. Adrenaline was injected intramuscularly, in doses of 0.1 (n=4), 0.2 (n=14), or 0.4 (n=7) mg for control subjects and 0.2 mg for patients, with the injection site massaged mildly. When propranolol (n=4) was administered, a priming dose of 5.0 mg was injected intravenously, followed by constant infusion at a rate of 0.08 mg/min for 20 min; adrenaline (0.1 mg) was injected 5 min after the start of the propranolol injection, Phentolamine (n=4) was administered in a 5.0 mg priming dose followed by infusion at a rate of 0.2 mg/min for 15 min before and 45 min after the injection of adrenaline (0.2 mg). When atropine (n=4) was used, subjects were given a subcutaneous injection of 0.5 mg atropine sulfate immediately before the adrenaline (0.2 mg) injection. Blood samples were taken at 5, 10, 15, 30, 45, and 60 min.

Assay procedures for cyclic AMP

The blood specimens were immediately mixed with a solution of EDTA (500 mmol/l) to make a final concentration of 10 – 30 mmol/l, enough for complete inhibition of cyclic nucleotide phosphodiesterase. After centrifugation, cyclic AMP in the supernatant was succinylated quantitatively and then submitted to microradioimmunoassay. Reagents used for the radioimmunoassay were obtained from a “Yamasa cyclic AMP assay kit” donated by Yamasa Shoyu Co. (Chiba, Japan). The statistical evaluation was made by Student's t test.

RESULTS

The plasma cyclic AMP values in normal subjects after an intramuscular injection of adrenaline at different doses are shown in Fig. 1, left. The injection of adrenaline caused a sharp increase in plasma cyclic AMP with a peak between 10 and 15 min; the peak level being dependent on the dose of adrenaline.

![Fig. 1. Plasma cyclic AMP responses to adrenaline in healthy subjects (left panel). Adrenaline, 0.1 (○), 0.2 (△) and 0.4 (□) mg per person, was injected intramuscularly into 4, 14, and 7 healthy adults respectively at time-0. Each point with a vertical line is the mean value ± SE. The middle and right panel show plasma cyclic AMP responses to adrenaline with simultaneous administration of propranolol (●), phentolamine (△), and atropine (□). The shaded areas represent responses (mean ± SE) of 4 (middle) and 14 (right) healthy subjects to 0.1 and 0.2 mg of adrenaline respectively.](image-url)
Plasma Cyclic AMP Response to Adrenaline

Table 1. Baseline concentrations of plasma cyclic AMP in healthy subjects and patients.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number of subjects</th>
<th>Plasma cyclic AMP (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>14</td>
<td>17.5 ± 1.1</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with spinocerebellar degeneration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>before phthalazinol</td>
<td>10</td>
<td>12.1 ± 1.2&lt;sup&gt;a&lt;/sup&gt;)</td>
</tr>
<tr>
<td>after phthalazinol</td>
<td>10</td>
<td>17.3 ± 2.2</td>
</tr>
<tr>
<td>with bronchial asthma</td>
<td>9</td>
<td>23.1 ± 3.3</td>
</tr>
<tr>
<td>with pulmonary emphysema</td>
<td>9</td>
<td>21.9 ± 1.0&lt;sup&gt;b&lt;/sup&gt;)</td>
</tr>
<tr>
<td>with diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dietary control alone</td>
<td>8</td>
<td>15.0 ± 1.5</td>
</tr>
<tr>
<td>insulin-dependent</td>
<td>8</td>
<td>15.6 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. <sup>a</sup>) Significantly different from control (P < 0.01). <sup>b</sup>) Significantly different from control (P < 0.05).

The increase in plasma cyclic AMP after the injection of adrenaline completely disappeared when propranolol was simultaneously injected (Fig. 1, middle), while it was not affected by simultaneous administration of phentolamine or atropine (Fig. 1, right).

The baseline concentration of plasma cyclic AMP was significantly lower in patients with spinocerebellar degeneration before phthalazinol treatment than in normal subjects (Table 1). The plasma cyclic AMP increase after adrenaline administration of these patients was also smaller than that of the healthy controls (Fig. 2, left), with the peak increase of 10.7 ± 2.3 Δpmol/ml that was significantly lower than that in the control group (23.8 ± 5.2 Δpmol/ml, P < 0.05).

One month after the initiation of the phthalazinol treatment these patients were re-investigated. Not only their clinical symptoms were found to have improved, but also the plasma concentration of cyclic AMP both before and after the adrenaline injection had increased to levels comparable to that of the normal subjects (Table 1 and Fig. 2, left).

Subjects with bronchial asthma exhibited a slightly and insignificantly higher concentration of plasma cyclic AMP than healthy subjects (Table 1). The plasma cyclic AMP increase after adrenaline administration of these patients was smaller than that of the healthy controls (Fig. 2, middle), with the peak increase of 11.0 ± 2.2 Δpmol/ml significantly lower than that in the control group (P < 0.05).

The baseline concentration of plasma cyclic AMP was significantly higher in patients with pulmonary emphysema than in healthy controls (Table 1). The plasma concentration of cyclic AMP in these patients reached the peak level more slowly than that in healthy controls after adrenaline administration, while there were no significant differences in the peak concentrations between these two groups (Fig. 2, middle).

There was no significant difference in the plasma concentration of cyclic AMP between diabetic patients and control subjects either before or after adrenaline injection (Table 1 and Fig. 2,
Fig. 2. Plasma cyclic AMP responses to adrenaline (0.2 mg/person) in patients with spinocerebellar degeneration, bronchial asthma, pulmonary emphysema and diabetes mellitus. The shaded areas represent responses of 14 healthy subjects to 0.2 mg of adrenaline injected as in Fig. 1. Ordinates show differences from the initial levels (Δpmol/ml plasma). The actual concentrations of plasma cyclic AMP before adrenaline administration are presented in Table 1. Vertical lines show SEM. Left panel: Patients with spinocerebellar degeneration (○) before phthalazinol treatment, ▲ one month after treatment with a daily dose of 300 – 600 mg phthalazinol). Middle: Bronchial asthma (●) and pulmonary emphysema (▲) patients. Right: Patients with diabetes mellitus (●) dietary control alone, ▲ insulin-dependent diabetes).

right). The plasma concentration of cyclic AMP in patients with insulin-dependent diabetes reached the peak level more slowly than that in diabetic patients with dietary control alone. But there was no significant difference in the cyclic AMP response between the patients of insulin-dependent diabetes and controls.

The patients and controls in this study were well age-matched except for the pulmonary emphysema cases. The controls and patients could not be matched by sex, but the males and females in each group responded similarly.

DISCUSSION

The adrenaline dose (0.2 mg/person, 3.3-4 μg/kg) employed in the present study is lower than the doses in the adrenaline tests adopted in previous reports\(^2, 3, 8-10\). Such a low dose was chosen because it causes a moderate, consistent, and short-lived increases in plasma cyclic AMP in healthy volunteers without any serious side effect. Adrenaline-induced increases in plasma cyclic AMP was almost totally abolished by propranolol but was not affected by phentolamine or atropine, indicating that the increase in plasma cyclic AMP in humans would reflect susceptibility of their β-adrenergic receptors in the response to administered catecholamines. Many hormones such as parathyroid hormone, glucagon, insulin, and ACTH are also known to increase plasma cyclic AMP. But they cannot be responsible for the cyclic AMP response to adrenaline, since propranolol suppressed the responses.

Adrenaline caused smaller increments in plasma cyclic AMP in patients with spinocerebellar degeneration or asthma than in healthy subjects. The finding is consistent with reports over the past decade that patients with asthma have a defective β-adrenergic effector system\(^8-11\). However, the mechanism for the patients' blunted responses might be different for the neurological and respiratory diseases. In patients with spinocerebellar degeneration, the plasma concentration of cyclic AMP was lower both before and after the injection of adrenaline. If it is assumed that the basal concentration of cyclic AMP is a reflection of endogenous catecholamine functions, the patients would appear to suffer from blunted responses to endogenous and exogenous catecholamines. Alternatively, endogenous catecholamines would be smaller in amounts in the patients than in the healthy subjects. In contrast, impaired cyclic AMP responses of patients with asthma were associated with slightly higher, rather than lower, basal levels than normal subjects. It is conceivable that more endogenous catecholamines might be available in these patients, to minimize bronchoconstriction, thereby raising the basal level of cyclic AMP and attenuating its susceptibility to the small amount of adrenaline used in this study. Alternatively, the previous daily medication with β-adrenergic agonist, though it was discontinued 3 days before the study, might be responsible for the higher basal value of plasma cyclic AMP. If such was the case, the administration of a β-adrenergic antagonist would normalized the basal level of plasma cyclic AMP; no antagonist was injected, however, due to its unfavorable effects on asthmatic patients. In the case of patients with emphysema, adrenaline caused normal increments in plasma cyclic AMP, although the basal level of cyclic AMP was higher than in healthy controls. The reason for
the different response patterns between the two respiratory diseases is not clear, but the susceptibility of β-adrenergic receptors in the response to administered catecholamines may be more attenuated in asthmatic patients than patients with emphysema.

There has been no specific therapy for symptoms of spinocerebellar degeneration. It was recently suggested that phthalazinol, a new cyclic AMP phosphodiesterase inhibitor, was very effective in reducing several symptoms of cerebellar ataxia. In this study, the phthalazinol treatment was very effective in restoring the normal plasma concentration of cyclic AMP either before or after the adrenaline injection; it also caused consistent improvement of several symptoms such as cerebellar ataxia of the trunk and extremities, dysphagia, dysarthria, nystagmus, etc.

In the case of diabetic patients, the plasma cyclic AMP response pattern was normal in patients with dietary control alone but somewhat different from normal in patients with insulin-dependent diabetes. It might be related to the fact that the insulin-dependent diabetic patients tended to manifest diverse symptoms of peripheral neuropathy, such as orthostatic hypotension, impotence, neurogenic bladder, and abnormalities of the pupil more frequently than the patients with dietary control alone.

It is necessary to determine whether a dose-response of plasma cyclic AMP to adrenaline as observed in normal volunteers is also seen in patients, since the response in patients may be different from the controls. However, we could not get consent for further studies from the patients.

Although the mechanism whereby the adrenaline-induced increase in plasma concentration of cyclic AMP is blunted in asthmatic patients and in patients with spinocerebellar degeneration is still unknown, adrenaline test described here would be a promising diagnostic means for detecting β-adrenergic dysfunctions in some diseases.

REFERENCES